Optimizing harvesting procedures of *Amaranthus hybridus* L. and *A. tricolor* L. under different watering regimes during hot and cool seasons in southern Mozambique

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Dissertation presented for the Degree of Doctor of Philosophy
in the Faculty of AgriSciences, Department of Agronomy, at
Stellenbosch University

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March 2017

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

Drought has been the major constraint for vegetable and food crop production in arid and semi-arid regions as is the case in southern Mozambique with a tropical dry savanna climate that is prone to droughts. In this vulnerable region, malnutrition associated with scarcity of vegetables imposed a serious constraint in the diet of rural communities. Rural communities are forced to use wild plants, such as amaranth, as a way to supplement their nutrition. Here Amaranth species grow naturally and the leaves are regularly collected manually to be consumed as tender greens. There is no evidence of grain consumption. Although few growers cultivate amaranth in small areas or in their gardens, the intensity and frequency with which the leaves are collected has not been tested yet. The production of multi-purpose amaranth, a C_4 plant widely distributed in the tropics and relatively drought-tolerant crop, offer a great potential to play a beneficial role in nutrition and food security.

Three field experiments with *Amaranthus hybridus* and *A. tricolor* repeated six times each (three during the rainy season and three in the dry season) were carried out during the period from December 2013 to October 2015 in Maputo. A randomized complete block design in a factorial arrangement was used in each experiment. Experiment one aimed to assess the vegetative growth, flowering, leaf and grain yields, as well as nutrient contents in leaves and grain of those species when subjected to watering regimes of 80%, 50% and 20% of total available water. The relationship between temperature and day length on the leaf yield and the time to flowering were also assessed (Chapters 3 and 4). The treatments were laid out in a 3 x 2 factorial arrangement with six replications. In experiments two (Chapther 5) and three (Chapter 6), under the same watering regimes and with the same species,

the vegetative growth, leaf yield and nutrient content were assessed as affected by harvesting intensity (plants topped by 25% and 50% of their heights) and harvesting frequency (every two weeks and every three weeks) respectively. In these two experiments, the treatments were laid out in a 3 x 2 x 2 factorial arrangement with three replications. Results from experiment one revealed that vegetative and reproductive growths were sensitive to soil water contents of 50% and 20% of total available water. However, the vegetative growth was less susceptible to water deficits that occurred in short intervals throughout the rainy season. Higher calcium and crude protein contents in the leaves were found at low water levels with the highest values obtained in *A. tricolor*. Temperature significantly affected the high leaf yield during the rainy season while day length had a noticeable influence on the low leaf yield during the dry season. The onset of flowering was determined by day length and minimum temperature with day length the most determining factor. The vegetative growth of both species showed similar behavior in response to different soil water and climate conditions over the year. The highest grain yield and harvest index was obtained in *A. tricolor* with an increase in minimum temperature which delayed flowering mainly when the day length was above 12 hours day^{-1} during the rainy season.

The results from experiment two and three indicated insufficient evidence to support the hypothesis that the combined effects of watering regimes and harvesting intensity, and watering regimes and harvesting frequency affect vegetative growth in both species. However, the vegetative growth of both species was tolerant to water deficit at 50% of total available water with successive cuttings. The best harvesting intensity and frequency found was 25% of their heights and two-week intervals respectively, since this frequency yielded more small and tender green leaves which

are preferred by the consumer. *Amaranthus hybridus* showed better performance and higher leaf yield compared to *A. tricolor* over the year. Results also revealed that the multiple harvests extend the vegetative growth phase which is an advantage for amaranth leaf production, especially under short days during the dry season. In plants harvested several times, the calcium and crude protein were not affected by watering regimes. However, the highest calcium and crude protein content in the leaves were obtained at final and first harvests respectively.

As a leafy vegetable, *A. hybridus* showed to have potential to become a suitable crop throughout the year and to supplement calcium and protein requirements in the diet of rural communities. It is recommended to be cultivated in the rainy season under rain-fed condition with supplemental irrigation and in the dry season as an irrigated crop at 50% of total available water. In both seasons, the leaf harvesting should be topping by 25% at 2 weeks intervals.

Uittreksel

Droogte is die belangrikste beperkende faktor vir voedselproduksie in ariede en semi-ariede gebiede soos in Suid- Mosambiek wat ‘n droë tropiese savanna klimaat het wat aan gereelde droogtes onderwerp word. In hierdie kwesbare gebied is wanvoeding as gevolg van ‘n gebrek aan voldoende groenteproduksie ‘n ernstige beperking in die diet van landelike gemeenskappe. Landelike gemeenskappe word dus gedwing om wilde plante soos amarante te verbruik om voedingsbehoeftes aan te vul. Amarantspesies groei wild hier en die blare word gereeld versamel om as vars groente geëet te word. Daar is geen getuienis dat amarantplante se saad gebruik word as voedsel nie. Alhoewel ‘n paar produsente anmarant in klein gebiede of in hulle tuine verbou, is daar nog geen navorsing gedoen om die optimum frekwensie en intensiteit van die oesproses van die blare te bepaal nie. Die produksie van die meerdoelige amarant, ‘n C_4 plant wat wydverspreid in die trope voorkom en ‘n redelike droogtebestande gewas is, toon groot belofte om ‘n groot rol te speel in voedselsekerheid en voeding.

Drie veldproewe met *Amaranthus hybridus* en *A. tricolor* wat ses keer herhaal is (drie keer gedurende die reënseisoen en drie keer tydens die droë seisoen) is uitgevoer gedurende die periode van Desember 2013 tot Oktober 2015 in Maputo. ‘n Volledig ewekansige blokontwerp is in alle proewe gebruik. Eksperiment een se doel was om die vegetatiewe en reproduktiewe groei sowel as die blaar- en graankwaliteit van die betrokke spesies te bepaal wanneer dit blootgestel is aan waterpeile van 80%, 50% en 20% van totale beskikbare water. Die verhouding tussen temperatuur en daglengte en die invloed daarvan op die blaaropbrengs en tyd tot blomvorming is ook ondersoek (Hoofstukke 3 en 4). Die behandelings is gereël as ‘n 3 x 2 faktoriaal uitleg met ses herhalings. In Eksperimente twee

(Hoofstuk 5) en drie (Hoofstuk 6), onder dieselfde waterbehandelings met dieselfde spesies, is die vegetatiewe groei, blaaropbrengs en nutriëntinhoud se reaksie op oesintensiteit (plante gesny met 25% en 50% van hulle hoogtes) en oesfrekwensie (elke twee weke en drie weke) onderskeidelik, gemeet. In hierdie twee eksperimente is die behandelings toegepas as 'n 3x2x2 faktoriaal gereëlde eksperiment met drie herhalings. Resultate van Eksperiment 1 het gewys dat vegetatiewe en reproduktiewe groei sensitief was vir grondwatervlakke van 50% en 20% van totale beskikbare water. Die vegetatiewe groei was egter minder vatbaar vir kort periodes van watertekorte wat gedurende die reënseisoene voorgekom het. Hoër kalsium en ruproteien vlakke in die blare het voorgekom by lae watervlakke en die hoogste waardes het voorgekom in *A. tricolor*. Die hoë blaarproduksie tydens die reënseisoen is betekenisvol beïnvloed deur temperatuur terwyl die heelwat laer blaarproduksie tydens die droë seisoen betekenisvol deur daglengte beïnvloed is. Die aanvang van blomvorming is bepaal deur daglengte en minimumtemperature met daglengte die mees bepalende faktor. Die vegetatiewe groei van beide spesies het dieselfde tendense getoon in reaksie op die verskillende grondwater en klimaatstoestande deur die jaar. Die hoogste graanopbrengs en oesindeks is waargeneem in *A. tricolor* met 'n toename in minimum temperature wat blomvorming hoofsaaklik onderdruk het wanneer die daglengte bo 12 ure per dag was tydens die reënseisoen.

Die resultate van Eksperimente twee en drie het nie genoegsame getuienis gelewer om die hipotese te ondersteun dat die gekombineerde effek van watervlakke in die grond en oesintensiteit, asook watervlakke en oesfrekwensie die vegetatiewe groei van beide spesies affekteer nie. Die vegetatiewe groei van beide die spesies was egter redelik verdraagsaam vir 'n watertekort van 50% van totale beskikbare water wanneer hulle herhaaldelik gesnoei is. Die beste oesintensiteit en oesfrekwensie is gevind wanneer die boonste 25% afgeknip is en tweeweekliks geknip is respektiewelik omdat hierdie betrokke oesfrekwensies en oesintensiteite meer klein en sagte blaartjies voortbring wat deur verbruikers verkies word. *Amaranthus hybridus* het beter presteer en meer blare geproduseer deur die twee jaar as *A. tricolor*. Die resultate het ook getoon dat meervoudige oeste die vegetatiewe groeifase verleng het wat voordelig is vir amarant blaarproduksie, veral tydens kort dae gedurende die droë seisoen. In plante wat ‘n paar keer agtereenvolgens geoes is, is die kalsium- en ruproteienvlakke nie deur die waterbehandelings beïnvloed nie. Die hoogste kalsium en ruproteienvlakke is egter in die blare gevind tydens die eerste en finale oesdatums respektiewelik.

Samevattend kan dus gesê word dat *A. hybridus*, as ‘n blaargroente, die potensiaal het om ‘n geskikte gewas te word wat deur die jaar produseer en die kalsium- en ruproteieninhoud kan bydra om die kalsium- en ruproteienvereistes in die diëte van landelike gemeenskappe te bevredig. Dit word aanbeveel om in die reënseisoen onder droëlandtoestande met bykomende besproeiing en in die droë seisoen as besproeide gewas teen 50% van totale beskikbare water verbou te word. In beide seisoen behoort blaaroes plaas te vind teen verwydering van 25% van die hoogte van die plant elke twee weke.

Acknowledgements

I would like to express my sincere gratitude and warm appreciation to a number of people and institutions who have made this work possible. I have to say that this study would not have been feasible without their encouragement and assistance. Amongst those who have directly or indirectly contributed to this study, I would like to mention the following people and institutions:

- ✓ Dr. Petrus Jacobus Pieterse and Dr. Sebastião Inácio Famba, my supervisors, for their guidance in all stages of the study, particularly their valuable suggestions in structuring the research and encouraging me throughout the course of my work.
- ✓ Mr. Alexandre Chaúque for providing the seed of *A. hybridus* used in the study.
- ✓ Mrs. Erminia Cossa (research technician), Mr. Jeremias Massimbe (student) and Mr. Zacarias Chaia (student) for their continuous support and valuable assistance with field trials and data collection.
- ✓ Mrs. Lucrécia Pereira, Mrs. Ilda Macunguel, Mrs. Belmira Paulo and Mrs. Rita Hafussene (research technicians); Mr. Moisés Mazive, Mrs. Adozinda Rafael, Mrs. Inês Honwana, Mr. Betuel Sigauque, Mr. Amós Nhamango, Mrs. Albertina Machavane, Mrs. Raquel Wate, Mr. Vasco Bila and Mr. Chadreque Missossa (field workers); Mr. Gervâncio Covele, Mr. Manuel Cossa, Mr. Nelson Carvalho, Mr. Jossefa Paruque and Mr. Dinis Colela (students) for assisting me with the field work and data collection.
- ✓ Mr. António Machava and his team for laboratory analyses.

✓ Dr. António Ribeiro for his constructive suggestions during the writing stage of this study.

✓ SHARE Project for granting the scholarship that made this study possible.

✓ ARC-Roodeplaat Vegetable and Ornamental Plant Institute, Pretoria, for providing the seed of *A. tricolor* used in the study.

✓ Faculty of Agronomy and Forestry Engineering, Eduardo Mondlane University, Maputo, for providing research facilities.

✓ National Research Fund of Mozambique (FNI) for the financial support to laboratory analyses.

I am grateful to all my friends and family for their support and friendship especially to whom had showed their confidence in me and financially contributed to my first degree in Agronomy. Last but not the least, I wish to express my thankful appreciation to Júlio Ribeiro and the late Maria Ester, my parents, whose upbringing I value so highly. I thankfully acknowledge all their support and friendship.

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Chapter 1

Introduction

1.1 Background

Drought has been the major constraint for food production in arid and semi-arid regions. Increased food production and productivity and ensured food diversity are priorities to improve food security and nutrition in southern Mozambique (RM 2011). The predominant farming systems are based on rain-fed agriculture and the smallholder farming is predominantly low input staple food production by means of manual labor with little or no mechanization, restricted to small areas and centered on the family workforce and needs (FAO 2005, Rutherfoord 2010). The production of crops such as maize, cassava and beans has shown some increase during the last two decades, yet the diet diversification is still inadequate for 50% of households (RM 2010). Despite that increase, the production of exotic vegetables such as tomato, lettuce, kale and cabbage as sources of minerals and vitamins is risky in the region due to low and erratic rainfall associated with high temperatures during the rainy season. As noted by Droogers et al. (2001) the potential for rain-fed crop production are almost zero for most of this region. Moreover, only 30% of 75,747 ha of the area equipped for irrigation are operational (INGC 2009), consequently the malnutrition associated with the shortage of vegetables in the human diet is a serious problem in rural communities (RM 2010).

Southern Mozambique lies between 20.57ºS and 26.51ºS latitudes, and 31.30ºE and 35.34ºE longitudes and is located on the east coast of Southern Africa edging the Indian Ocean. It is characterized by a tropical dry savanna (BS) in most of its interior, according to the Köppen classification (INGC et al. 2003), and prone to droughts (INGC 2009). In terms of rainfall and temperature, two distinct seasons are recognized, the rainy and hot season (from October to March) and the dry and cool season (from April to September). The average annual rainfall varies from 500 to 600 mm inland and 350 mm in the driest interior areas. The mean annual temperature varies from 23º to 26ºC with average maximum and minimum temperatures of 30ºC and 19ºC respectively. The altitude is less than 200 m above sea level in 90% of the terrain, and the mean water capacity of soil is less than 100 mm in most of the region (Reddy 1986). Soil fertility is low except in floodplains (INGC 2009). These conditions make the production of rain-fed food crops very difficult or even impossible. Therefore, many rural communities use wild plants, such as amaranth, as a way to supplement their nutrition and food security.

1.2 Motivation and problem identification

In recent years, several studies have addressed the reduction of food scarcity in vulnerable areas in Mozambique. The most relevant studies are as follow: (1) the assessment of the potential of rainwater harvesting technologies on maize yield and the evaluation of maize and cowpea yields on a variation of planting basins named the Zai Pits System (Ncube et al. 2010); (2) the effect of conservation agriculture focusing on maize yield and soil water balance in particular in order to be adopted by smallholder farming under rain-fed low input crops (Famba 2011); (3) the productivity performance of 44 sweet potato varieties cultivated under irrigation and drought (rain-fed) conditions in the South of Mozambique (Maquia et al. 2013); (4) comparison of performance of four cowpea landraces under drought conditions (Martins et al. 2014); (5) the assessment of the variability of 136 cowpea genotypes in terms of drought tolerance (Chiulele et al. 2011). Although amaranth species occur and is commonly used, they are seldom seen cultivated. There are no studies in Mozambique on the agro-production of these species as a leafy vegetable or as grain crop.

Amaranth (*Amaranthus* spp.) is considered as being one of the oldest food crops in the world. In pre-Columbian times amaranth grain was used as a staple food and in religious ceremonies during the Inca and Aztecs Dynasties of South America and Mexico (National Research Council 1984). In the 1500s, the Spanish conquistadors attempted to suppress the use of amaranth in religious ceremonies leading to the decline of amaranth production (Sauer 1967). However, the amaranth survived as a grain crop and was produced in scattered areas of Central and South America (National Research Council 1984). Meanwhile amaranth species were introduced in Europe as an ornamental plant and later in Africa and Asia where they were used mainly as green vegetable (Sauer 1993). In the last ten years, amaranth has been seen as a potential crop not only to improve the nutrition but also to be a source of income for families especially in sub-Sahara Africa. This has been mentioned by several authors, among them are Materechera and Medupe (2006), Olufolaji et al. (2010), Bello et al. (2011), Masarirambi et al. (2012), Awe and Osunlola (2013).

The multi-purpose amaranth is a C_4 plant widely distributed in the tropics (Ebert et al. 2011), a relatively drought-tolerant crop (Liu and Stützel 2004, Olufolaji et al. 2010) and can be successfully cultivated in arid and semi-arid regions (Schahbazian et al. 2006). As a leafy vegetable, amaranth species can be produced in regions and seasons where other crops are inaccessible, since they grow in poor soil conditions and tolerate temperatures up to 40ºC (Ebert et al. 2011). In addition, their leaves contain high quality protein with relevant levels of lysine (Andini et al. 2013) and a significant amount of iron, calcium, and vitamins A and C which are required by human beings (Allemann et al. 1996, Akubugwo et al. 2007). The grain contains high quality proteins, particularly, lysine amino-acid as well (Pospišil et al. 2006, Venskutonis and Kraujalis 2013). Thus amaranth species appears to be a potential crop to improve the diet of rural communities in dry semi-arid areas of southern Mozambique.

According to Silva et al. (2004), *Amaranthus thunbergii*, *A. caudatus, A. dubius, A. graecizans, A. hybridus, A. spinosus* and *A. viridis* occur in Mozambique. They grow naturally and in cultivated fields emerge as a weed with a pronounced vegetative growth in the rainy season. These plants are generally protected to be consumed as tender greens. The leaves are regularly collected manually leaf by leaf or by cutting the top portion of main stems and thereafter by cutting side-shoots allowing repeated harvestings. There is no evidence of grain consumption. Although few growers cultivate amaranth in small areas or in their gardens, the intensity and frequency with which the leaves are collected has not been tested yet. The harvesting procedures are key issues in the productivity of most amaranth crops. The harvesting frequency and the portion of leaves and buds left after cutting, and the period that the plant can withstand drought conditions without losing productivity are crucial issues in arid and semi-arid regions. This study intends to contribute to knowledge about the sustainable production of nutritive amaranth species all year round to enhance nutrition and food security in southern Mozambique. Two amaranth species were selected for the study, *A. hybridus,* one of the species that occurs naturally in southern Mozambique (Silva et al. 2004) and *A. tricolor* considered as one of superior species as a leafy vegetable (Daloz and Munger 1980) and well known in Asia (Grubben 2004) but new in Mozambique.

1.3 Objectives

1.3.1 General objective

The general objective of this study was to assess the effect of harvesting procedures and watering regimes on growth, yield and quality of *A. hybridus* and *A. tricolor* in southern Mozambique.

1.3.2 Specific objectives

To achieve the general objective, the following specific objectives were set out:

1. (a) to assess the response of *A. hybridus* and *A. tricolor* to different watering regimes in terms of vegetative growth and leaf nutritional content during the rainy and dry seasons in southern Mozambique and

   (b) to ascertain relationships between temperature and day length on leaf yield of both species (Chapter 3);
2. (a) to evaluate the effect of watering regimes on flowering, grain yield and grain crude protein content of *A. hybridus* and *A. tricolor* during the rainy and dry seasons in southern Mozambique and

   (b) to evaluate the effect of the relationship between day length and minimum temperature on the time to flowering for both species (Chapter 4);
3. to assess the effect of harvesting intensities and watering regimes on the vegetative growth, leaf yield and leaf nutritional content of *A. hybridus* and *A. tricolor* during the rainy and dry seasons in southern Mozambique (Chapter 5);
4. to assess the effect of harvesting frequencies and watering regimes on vegetative growth, leaf yield and leaf nutritional content of *A. hybridus* and

A. tricolor during the rainy and dry seasons in southern Mozambique (Chapter 6).

The hypotheses are as follows:

1. (a) *Amaranthus hybridus* and *A. tricolor* tolerate moderate water deficit in terms of leaf yield and nutrient contents.

   (b) Low temperature and short days decrease leaf yield in both species (Chapter 3);
2. (a) the flowering delay and grain yield and grain crude protein content decrease under moderate water deficit in both species.

   (b) Long days and high minimum temperature delay flowering in both species (Chapter 4);
3. Light harvesting intensities reduce the effect of drought on the vegetative growth, leaf yield and leaf nutritional content of *A. hybridus* and *A. tricolor* (Chapter 5);
4. High harvesting frequency reduces the effect of drought on vegetative growth, leaf yield and leaf nutritional content of *A. hybridus* and *A. tricolor* (Chapter 6).

1.4 Dissertation outline

After this introduction, Chapter 2 is the literature review focused on the response of *A. hybridus* and *A. tricolor* to drought conditions and leaf harvesting intensities and frequencies. This dissertation is written as stand-alone publications (Chapters 3, 4, 5 and 6) and consequently some duplication may occur particularly on Introduction, Discussion and Material and Methods of each publication. It is noteworthy here that the data used in Chapter 4 (reproductive phase) were collected during the research study of Chapter 3 (vegetative phase) and the data used in Chapter 5 and 6 were gathered separately. All publications were prepared according to the directions of the South African Journal of Plant and Soil. At the time that this dissertation was handed in for examination, Chapter 3 (Vegetative growth *of A. hybridus* and *A. tricolor* under different watering regimes in different seasons in southern Mozambique) has been accepted for publication by the South African Journal of Plant and Soil (in press) and Chapters 4, 5 and 6 will be submitted to different international and local journals.

The overall summary discussions, conclusions and recommendations of this study are made in Chapter 7. An appendix is included with results of Analysis of Variance and Covariance of data used in Chapters 3, 4, 5 and 6.

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Chapter 2

Literature Review

2.1 Introducing *Amaranthus hybridus* and *Amaranthus tricolor*

Amaranthaceae, also known as the amaranth family, embraces some 65 genera and 900 species, most of them natives of tropical and sub-tropical regions of Africa, and Central and South America (Cwynar 2004). Many of them are however considered as being almost cosmopolitan in distribution and often introduced (Steentoft 1988). The *Amaranthus* genus of this family comprises some 70 species predominantly originated from America and a few from other continents (Costea and DeMason 2001). The taxonomy of this genus is rather difficult to be accurately described owing to its high phenotypic variability and hybridization among the species. The *Amaranthus* genus is currently grouped into three subgenera (Mosyakin and Robertson 1996); each subgenus is differentiated by sections and frequently by subsections. According to them, the three subgenera are: *Amaranthus* subgen. *Acnida* (L.) Aellen ex K. R. Robertson, *Amaranthus* subgen. *Amaranthus* and *Amaranthus* subgen. *Albersia* (Kunth) Gren. & Godr..

Amaranth is a multi-purpose plant used worldwide as a grain crop or leafy vegetable for food, forage for animals and as ornamental. *Amaranthus caudatus*, *A. cruentus* and *A. hypochondriacus* are species domesticated for grain production (Trucco and Tranel 2011), also known as pseudo-cereals. The most common amaranth species produced as leafy vegetables are *A. blitum, A. cruentus, A. dubius, A. spinosus, A. tricolor* and *A. viridis* (Ebert et al. 2011). In addition to, Das (2012) reported as weeds the following amaranth species: *A. spinosus*, *A. viridis*, *A. retroflexus*, *A. graecizans*, *A. dubius*, and *A. hybridus*. Although some amaranth species have been domesticated for a specific end use, they can be cultivated for different purposes. For instance, *A. cruentus*, previously described as a grain type, is also used as a leafy vegetable, fodder or ornamental (Grubben 2004a).

Amaranthus hybridus, native of America, belongs to subgenera *Amaranthus* sect. *Amaranth* subsect. *Hybrida* Mosyakin & K. R. Robertson (Mosyakin and Robertson 1996). In the 18th century, *A. hybridus* arrived in the Mediterranean region and later dispersed and became a naturalized weed in Eastern Asia, Australia and South Africa (Sauer 1967). This species is an erect, branched annual herbaceous plant which is referred to as wild relatives of the grain crops (Trucco and Tranel 2011). It is generally used as a leafy vegetable (Mepha et al. 2007, Mobina and Jagatpati 2015) and forage (Sleugh et al. 2001). Although it is well known as a weed, it is also a commercial leafy crop cultivated in some African countries such as Nigeria (Mofunanya et al. 2015), Burkina Faso (Ibrahim et al. 2012) and Côte d'Ivoire (Agbo et al. 2012). In Mozambique, the occurrence of *A. hybridus* is reported in Maputo (South), Tete (Central) and Lichinga (North) as an alien species (Silva et al. 2004, Kew Royal Botanic Gardens n.d.).

Amaranthus tricolor, probably from tropical Asia, belongs to subgenera *Albersia* sect. *Pyxidium* Moquin in DC (Mosyakin and Robertson 1996) and is an erect, strongly branching annual herbaceous plant of which the wild ancestor is unknown (Grubben 2004b). As a weed it does not occur abundantly like *A. hybridus* but it is the major leafy vegetable cultivated in South and Southeast Asia (Grubben 2004b, Ebert et al. 2011). It is also grown worldwide as ornamentals (Grubben 2004b). Its cultivation as a commercial vegetable has been reported in east and southern African countries such as Benin, Nigeria, Kenya and Tanzania, however with little economic significance (Grubben 2004b). Although there is no indication that *A.*

tricolor occurs naturally in Mozambique, the Collection of Flora Zambesiaca displays a specimen of this species as being “ornamental in the municipal garden of Maputo, 1945” (Kew Royal Botanic Gardens n.d.).

2.2 Amaranth species as affected by climatic conditions

The response of amaranth species, particularly *A. hybridus* and *A. tricolor*, to air temperature, sunlight intensity and day length is the topic of this section. Associated to this topic is how to take advantage of these environmental factors that regulate the physiological process to benefit biomass and grain production. Amaranth is a C_4 plant which means it is capable to capture and fix carbon dioxide (CO_2), convert it to a first stable product named oxaloacetic acid, a four-carbon acid, by phosphoenolpyruvate carboxylase during the initial step of the photosynthesis process (Hopkins 1999, Sage 2004). Although C_4 plants, also called tropical or warm season plants, are often associated with arid tropics, subtropics and warm temperate zones, these plants also occur in habitats varying from boreal zone, deserts and nutrient-depleted to fertile soil conditions (Christin and Osborne 2014). Sage (2004) suggested that the C_4 photosynthesis is an adaptation to any environmental factor that increases photorespiration and reduces carbon balance such as heat, drought, salinity and low CO_2, and even flooding which can stimulate photorespiration in certain situations. The evolution of C_4 plants in environments where carbon dioxide is reduced and temperature is high resulted in them being more efficient in using carbon dioxide, increasing the ratio of carboxylation to oxygenation reactions and hence lowering the rate of photorespiration (Ehleringer et al. 1991). This gives the amaranth species a great capacity of adaptation in tropical and sub-tropical regions where it can tolerate temperatures up to 40ºC (Ebert et al. 2011) even in warm temperate zones where the night temperature is not lower than 15°C (Grubben 2004b).

The vegetative growth of *A. hybridus* and *A. tricolor* is strongly affected by temperature. In an experiment carried out in controlled-environment growth chambers with *A. hybridus* at three day/night temperature regimes of 25/20, 30/25 or 35/30 °C, Fawusi et al. (1983) found that, although the plants consistently produced greater stem lengths at temperatures of 30/25 °C and 35/30 °C day/night, the highest leaf area and greatest shoot dry weights were achieved at 30/25 °C day/night. Other experiments with *A. tricolor* under greenhouse conditions showed that the best results in terms of plant height, number of leaves and biomass were obtained at mean air temperatures of 28 and 29 ºC and sunlight intensities of 1240 and 1257 µmol m^{-2} S^{-1} when compared with 18, 19, 21, 24 and 25 ºC, and 850, 975, 1065, 1206 and 1208 µmol m^{-2} S^{-1} respectively (Khandaker et al. 2009). These results were attributed to high rates of photosynthesis and respiration since *A. tricolor* is a C_4 plant. In *A. tricolor*, peak photosynthetic rates were achieved at 35 ºC and an irradiance of 1.7-1.8 mmol m^{-2} S^{-1} (Lin and Ehleringer 1983). In relation to grain yield, Modi (2007) found a significant reduction in grain yield of *A. hybridus* and *A. tricolor* plants when the temperature increased from 27/21 °C day/night to 33/27 °C or dropped to 21/15 °C. This reduction under hot conditions (33/27 °C) as suggested by the author is a consequence of excessive temperature for growth and less transferred assimilates to the grain.

Some amaranth species have been reported as photoperiod-sensitive for flower induction (Huang et al. 2000, Grubben 2004b, Ebert et al. 2011). The onset of flowering for a quantitative short day plant is basically determined by the day length;

they initiate flowering when day lengths are shorter than 12 hours (Ebert et al. 2011). This was confirmed by Huang et al. (2000) where *A. retroflexus*, a quantitative short-day specie, took longer to reach the reproductive stage when exposed to 14 and 16 hours photoperiods compared to 8, 10 and 12 hours. However, high night temperatures (> 22 °C) may delay flowering in short day plants (Erwin 2005). According to Grubben (2004b), *A. tricolor* is a quantitative short day plant. Although day length is related to grain production, it can also affect the vegetative growth, since the grain development becomes the dominant sink (Hopkins 1999). Whitehead et al. (2002) found a significant reduction in *A. tricolor* vegetative growth with a decrease in day length and temperature. However, *A. hybridus* is considered a day neutral plant meaning the flowering is not dependent on photoperiodism (Mattson and Erwin 2005).

2.3 An overview of plant drought stress

2.3.1 Plant responses to drought: effects and survival mechanisms

Drought is one of the environmental factors that cause a considerable decline in crop yields. This section analyse how the plants survive and react to drought stress. It is well known that drought reduces plant growth and development, and consequently decrease the crop yield. The water shortage cause a decrease in cell turgor, cell division, cell enlargement and differentiation (Hsiao 1973, Blum 2011, Akinci and Lösel 2012) along with a reduction in nutrient uptake, photosynthetic activity (Akinci and Lösel 2012), radiation-use efficiency and harvest index (Earl and Davis 2003).

Plants can sense drought stress and develop appropriate responses with adapting growth and development to endure it. Physiological and morphological responses occur in plants in order to survive adverse conditions, or specific mechanisms are enhanced to avoid stress conditions (Akinci and Lösel 2012). According to Lisar et al. (2012), drought tolerance is the ability of a plant to continue with its functions at low tissue water potentials. Akinci and Lösel (2012) stated that all plants which improve its growth at limited water are considered drought tolerant. It can be displayed in different degrees. To cope with the drought, tolerant plants develop specific physiological, biochemical, and molecular adaptive mechanisms (Akinci and Lösel 2012, Lisar et al. 2012). The severity and duration of drought stress associated with the plant's ability to develop these adaptive mechanisms have a strong influence on survival of the plant (Blum 2011, Akinci and Lösel 2012).

One of those mechanisms at molecular level includes accumulation of specific proteins. The water stress-specific proteins are synthesized in many plants and its accumulation in leaves to act as osmoprotectants for osmotic adjustment in response to water stress (Akinci and Lösel 2012, Lisar et al. 2012). However, a reduction in protein synthesis might occur depending on the level of stress and the plant type. Some cases of reduced soluble proteins caused by water stress mentioned in literature are in *Zea mays* (Jabasingh and Saravana Babu 2014), *Cynodon dactylon* (Barnett and Naylor 1966) and *Helianthus annuus* (Rodríguez et al. 2002). Proline is a proteinogenic amino acid that is often related with water-stressed plants. Proline accumulated in the cell, act as osmoprotectants, can stabilize cellular homeostasis, protects cell membranes and the protein integrity enhance the activities of different enzymes during the stress (Szabados and Savoure 2010). Osmotic adjustment is another crucial mechanism adopted by plants to maintain cell turgor and leaf hydration at low leaf water potential (Blum 2011).

The changes in the molecular and physiological mechanisms make plants morphologically adaptable to water deficits reducing photosynthesis and resulting in lower biomass yields (Lisar et al. 2012). Morphological change that occur in plants comprise reduction in plant height, internode length, leaf number, leaf size and total leaf area (Anjum et al. 2011, Akinci and Lösel 2012). Some plants can maintain the same leaf number but they are smaller as a result of the organ differentiation which seems to be more resilient to water deficit than expansion growth (Blum 2011). Flower production and grain filling are also negatively affected by physiological changes; smaller and fewer grains are generally produced (Anjum et al. 2011). According to Blum (2011) the flowering and reproduction stages of plants are the most susceptible to drought compared to the vegetative stages. However, the timing of the drought stress is extremely crucial for grain filling (Plaut 2003).

2.3.2 Amaranth species responses to drought stress

How amaranth species, particularly *A. hybridus* and *A. tricolor*, respond to drought is the topic of this section. Previous studies on physiological response in water-stressed amaranths have shown that proline build up in *A. dubius*, *A. cruentus* (Ferrarotto 2003), *A. hybridus* (Slabbert and Krüger 2004, Umebese et al. 2009), *A. tricolor* and *A. hypochondriacus* (Slabbert and Krüger 2004). The adaptive role of proline was not established in those studies. However, this proline accumulation provides a good indicator of plant water status during and after a stress period of water deficit (Ferrarotto 2003, Slabbert and Krüger 2004). Proline also seems to stabilize cellular structures during severe water stress in *A. hybridus* and *A. tricolor* in order to minimize the damage caused by dehydration (Slabbert and Krüger 2004). Umebese et al. (2009) found that the proline accumulation by salicylic acid in stressed plants of *A. hybridus* increase the capacity of plants to absorb water from the soil.

According to Slabbert et al. (2004), *A. hybridus* and *A. tricolor* develop mechanisms to help avoid desiccation under severe drought conditions. One of these is the adjustment of leaf size in order to have better control of the water use. The same authors also mentioned that the leaf area, relative water content, cell membrane stability and leaf water potential of those species recovered in a short period of time after restoring leaf hydration. Liu and Stützel (2004) found in their study a conservative water balance (water loss and water uptake) between plant organs in *A. tricolor*, *A. blitum* and *A. cruentus* under drought conditions. These results are particularly relevant in areas where amaranth species are cultivated as vegetable crops under rain-fed conditions. The recovery of grain yield and harvest index was also observed in plants of *A. cruentus* under drought conditions up to inflorescence formation (Mlakar et al. 2012).

Studies under greenhouse conditions and uninterrupted drought stress demonstrated that the plant height, leaf number, leaf area and leaf yield of *A. hybridus* decreased with reducing irrigation levels from 85% to 40% of field capacity (Masarirambi et al. 2012). In *A. tricolor*, the same parameters decreased only at wilting point (Singh and Whitehead 1992). With regard to reproductive stage, a significant reduction was found in *A. cruentus* grain yield under permanent water stress condition but the harvest index was similar to those under constant and adequate water supply (Mlakar et al. 2012). A study conducted in a screen house and in the field showed completely different results, the grain yield of *A. cruentus* was higher under moderate water stress compared to severe and unstressed conditions (Olufolaji et al. 2010).

2.4 Harvesting procedure

This section analyse leaf harvesting methods used in amaranth plants and why they are used. Amaranth species as vegetables are usually harvested once by uprooting or cutting at the ground level, or several times by repeated leaf cutting (Ebert et al. 2011). Plants of *A. tricolor* that are harvested only once about a month after sowing is the most common harvesting method used by commercial growers but sometimes a second harvest from the re-growth of the smallest plants cut at ground level three weeks later are also carried out (Grubben 2004b). According to Ebert et al. (2011), the reason for that is because *A. tricolor* have a fast growth and is quick maturing. However, Bello et al. (2011) found that plants of *A. cruentus* harvested several times (multiple harvests) are more productive than plants harvested once. Apart from the high productivity, multiple harvests may also alleviate the drought effect on leaf yield as in the case of *A. cruentus* (Diwani and Janssens 2001).

Different ways of leaf collection are often mentioned in literature to describe multiple harvests: repeated cutting (National Research Council 1984, Grubben 2004b, Ebert et al. 2011), topping (Mnzava and Masam 1985, Norman and Shongwe 1993) and picking off individual leaves (Maundu and Grubben 2004). Multiple harvests is preferred because it allow repeated harvesting providing thus a continuous supply of tender green leaves to be consumed and it suppress bolting (Ebert et al. 2011). However, the first and consequent cutting height (harvesting intensity), and the cutting frequency (harvesting frequency) are the two crucial factors of harvesting procedure which can be manipulated to obtain high leaf productivity in most amaranth crops. In general, the amaranth plants are ready for the first cut a month after transplanting or seeding (Mnzava and Masam 1985, Norman and Shongwe 1993, Bello et al. 2011, Ebert et al. 2011).

2.4.1 Harvesting intensity

Having considered the harvesting procedure, it is logical to look at the intensity at which the plants are harvested. Which cutting heights are recommended for amaranth species to improve leaf productivity is the topic of this section. The leaf yield reaction depends on the portion of leaves and buds left behind for re-growth (Mnzava and Masam 1985). Norman and Shongwe (1993) reported higher leaf yield with 15 and 20 cm initial cutting height compared to 10 cm in *A. hybridus*. In *A. cruentus*, the best cutting height found was at 20 to 25 cm (Grubben 1976). Mnzava and Masam 1985 also found 20 cm initial cutting height as most suitable for *A. cruentus* compared to 3, 10 and 15 cm. In an experiment conducted under pot and field conditions, the productivity of *A. cruentus* was consistently improved with 4 t ha^{-1} of organic fertilizer applied to plants cut at 20 cm above soil surface compared to 0, 2 and 6 t ha^{-1} and 10 and 15 cm (Akanbi et al. 2009). Under hydroponic conditions, Ribeiro and Combrink (2006) found the highest leaf yield of *A. tricolor* in plants growing at an electrical conductivity (EC) of 4 mS cm^{-1} using both cutting heights of 25% and 50% of plant height; however, 25% was the best cutting height for leaf yield when the plants were grown at a high EC level of 8 mS cm^{-1}. These results therefore clearly show that less destructive cutting heights were beneficial for amaranth species probably due to more leaf and buds remaining on the stem consequently resulting in a high level of auxin for re-growth as argued by Mnzava and Masam (1985).

2.4.2 Harvesting frequency

A further and equally important consideration is the frequency at which the leaves of amaranth species are generally harvested. The interval between two leaf cuttings is crucial for the final leaf yield since the leaves and buds left behind need time to accumulate dry matter. To achieve that, repeated cuttings every 2-3 weeks are recomended by Ebert et al. (2011). The highest shoot and leaf yields as well as leaf to stem ratio were achieved in *A. hybridus* plants harvested every 2 weeks compared to every 3 weeks (Norman and Shongwe 1993). Under different fertilization regimes, the effect of four harvesting frequencies (every week, 2 weeks, 3 weeks and no cutting) was studied by Materechera and Medupe (2006) on *A. hybridus* growth during two seasons (winter and summer). The results revealed that plants from weekly harvests produced more leaves but the leaves had no marketable size and yielded low dry matter in all the fertilization regimes. However, the harvest frequency of two weeks was recommended because it showed an increase in number of leaves and leaf dry matter produced per plant. Grubben (1976) concluded in his experiment that cutting every three weeks was better than every two weeks in *A. cruentus*.

2.4.3 Combined effect of harvesting procedure and drought stress

In the final analysis, it is argued that the harvesting procedure (intensity and frequency) may improve the leaf productivity in plants of amaranth species when submitted to drought stress. There is scarce literature on the combined effect of harvesting procedure and drought stress on the productivity of amaranth species. Nevertheless the results of two studies were used as an indicator for the use of harvesting intensity and frequency to minimize the effect of limited water on the leaf dry mass.

The first is the study by Diwani and Janssens (2001) where the plants of *A. cruentus* were submitted to multiple leaf harvests to reduce the drought effect on the leaf dry mass accumulation. The second is by Ribeiro and Combrink (2006) where the plants of *A. tricolor* were submitted to a high EC level of 8 mS cm^{-1}, under hydroponic conditions, to restore leaf yield to levels equal and superior to no-stressed (1 and 2 mS cm^{-1}) plants with a less destructive cutting of the 25% cutting height. Although this result cannot be extrapolated to the case of water-stressed plant, it might indicate that the frequent removal of leaves may also minimize the effect of limited water, since one of the effects of saline conditions is to reduce the uptake of water by the plant.

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Chapter 3

Vegetative growth of *Amaranthus hybridus* and *Amaranthus tricolor* under different watering regimes in different seasons in southern Mozambique

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Accepted for publication by the South African Journal Plant and Soil (in press)

Abstract

Drought tolerant crops with high nutritional value such as amaranth leafy vegetables have the potential to improve the diet of rural communities in dry semi-arid areas of southern Mozambique. A study was conducted aiming to: (a) assess the vegetative growth and leaf nutritional content of *Amaranthus hybridus* and *A. tricolor* when subjected to different watering regimes (80%, 50% and 20% of total available water) during the rainy and dry seasons, and (b) ascertain the relationship between temperature and day length affecting the leaf yield of the two species. Six field trials were conducted in a randomized complete block design with 2 x 3 factorial lay-out with six replications in Maputo. The vegetative growth was sensitive to soil water at 50% and 20% of available water. However, the leaf and side-shoot number, leaf area and yield were less sensitive to short interval water deficits during the rainy season. Leaf calcium and crude protein content were higher at low water levels. Leaf yield was significantly affected by temperature and day length, where total leaf yield increased during the rainy season, and decreased during the dry season. Both species have the potential for sustainable leaf production during rainy and dry seasons.

Keywords: amaranth, day length, leaf yield, temperature, watering regime

Introduction

Amaranth crops (*Amaranthus* spp.) have the potential to play a significant role in food security and nutrition in Mozambique. Amaranth is a C_4 dicotyledonous plant that can be produced in arid and semi-arid regions (Schahbazian et al. 2006), in poor soil conditions with low soil water levels (Ebert et al. 2011). Amaranth species have been reported as relatively drought-tolerant crops by Liu and Stützel (2004) and Olufolaji et al. (2010), and some of the species are photoperiod-sensitive, and initiate flowering when day lengths are shorter than 12 hours (Ebert et al. 2011). Amaranth leaves are a good source of high quality protein with relevant levels of lysine (Andini et al. 2013) and also contribute significantly to iron, calcium and vitamin A and C requirements of humans (Allemann et al. 1996, Akubugwo et al. 2007).

Southern Mozambique is characterized by a tropical dry savanna (BS) in most of its interior, according to Köppen classification (INGC et al. 2003). In terms of rainfall and temperature, two distinct seasons over the year are recognized, the rainy and hot season (from October to March) and the dry and cool season (from April to September). The longest day length has 13.69 hours sunlight in December and the shortest 10.58 hours in June. The predominant farming systems in the area are based on rain-fed agricultural production.

Malnutrition due to scarcity of vegetables in diets is a serious problem in many rural communities (RM 2010). During the rainy season the production of exotic vegetables such as tomato, lettuce and cabbage, is very challenging in this region due to low and erratic rainfall amounts coupled with high temperatures. Consequently, drought tolerant crops have been a priority to improve food security and nutrition in these vulnerable areas (INGC 2006). Amaranth production may help to minimize the malnutrition problem.

In Mozambique, amaranth plants grow naturally or in cultivated fields as a weed and the leaves are collected to consume as tender greens. There are few growers that produce amaranth in small areas or home gardens. According to Silva et al. (2004), *Amaranthus hybridus* grows in the southern and central areas of Mozambique. There is no indication that *A. tricolor* grows naturally in Mozambique, however it is well known in Asia (Grubben 2004) and is also considered one of the superior species for use as vegetables (Daloz and Munger 1980). Several studies described the vegetative growth responses of *A. hybridus* and *A. tricolor* to water stress under protected conditions such as greenhouse or pot experiments (Singh and Whitehead 1992, Liu and Stützel 2004, Masarirambi et al. 2012, Jomo et al. 2015), but information describing these species under field conditions is scarce. The production of the amaranth species as a vegetable crop is new in Mozambique, particularly *A. tricolor*. The vegetative growth is strongly affected by temperature in both species (Fawusi et al. 1983, Khandaker et al. 2009) and also by day length in *A.*

tricolor (Whitehead et al. 2002). *Amaranthus tricolor* require short days for flowering (Grubben 2004) while *A. hybridus* flowering is not dependent on photoperiod (Mattson and Erwin 2005).

In order to contribute to a sustainable all year round production of nutritive leafy vegetables, this study was conducted under field conditions with the following objectives: (a) to assess the response of *A. hybridus* and *A. tricolor* to different watering regimes in terms of vegetative growth and leaf nutritional content during the rainy and dry seasons in southern Mozambique; and (b) to ascertain relationships between temperature and day length on leaf yield in both species.

Material and methods

Six on-station field trials were carried out in Maputo (25°57′ S, 32°34′ E; 60 m above sea level) at the experimental station of the Faculty of Agronomy and Forestry Engineering (FAEF), Eduardo Mondlane University. Three trials took place during the rainy seasons: 15 January to 30 March 2014 (RS1), 23 September to 30 November 2014 (RS2), and 18 February to 27 April 2015 (RS3), and the other three during the dry seasons: 14 April to 30 June 2014 (DS1), 14 May to 20 July 2015 (DS2), and 11 August to 8 October 2015 (DS3). *Amaranthus tricolor* seeds used for these trials were obtained through the ARC-Roodeplaat (South Africa) and *A. hybridus seeds* were collected in March 2013 from a producer field in Palma district (Northern Mozambique) and later multiplied in the fields of the FAEF. The seedlings were germinated and grown in seedling trays filled with Hygrotech Seedling Mix in a net-covered house for 29, 36, 29, 27, 31 and 39 days before transplanting for RS1, RS2, RS3, DS1, DS2 and DS3 respectively. Nitrogen (N) was applied at a rate of

14.4 kg ha^{-1} using urea (46% N) at 14 and 21 days after sowing. Consequently, twenty seedlings were transplanted into a field plot spaced at 25 x 35 cm. Each plot covered a small area of 1.75 m^2 (1.40 x 1.25 m).

The soil of the experimental site is a sandy soil. Some physical and chemical characteristics of the soil are shown in Table 3.1. The field fertilizer management procedure started three days before transplanting with a basal application of 35 kg ha^{-1} of each nutrient: nitrogen, phosphorus and potassium using a mixture of urea, $NPK_{(12:24:12)}$ and potassium sulphate at a rate of 38, 146, and 35 kg ha^{-1} respectively. Top dressing with 35 kg N ha^{-1} using urea at a rate of 75 kg ha^{-1} each was split into three applications, 15, 30 and 45 days after transplanting (DAT), except in RS1 and DS1 seasons where only two split applications (20 and 40 DAT) were done at the same rate of 75 kg ha^{-1}.

Table 3.1: Some soil physical and chemical characteristics at the experimental station of Eduardo Mondlane University where the amaranth trial was carried out

Depth (cm)	Texture sand (%)	PWP	FC	TAW	$EC_{(1:2.5)}$ (mS cm^{-1})	pH_{H2O}	Organic matter (%)
		(mm)					
0-20	92.7	10.1	22.4	12.3	0.11	6.64	0.48
20-40	91.8	10.1	24.2	14.1	0.07	7.02	0.17

PWP, permanent wilting point; FC, field capacity, TAW, total available water; EC, electrical conductivity

Mean temperature per day during the trial periods were recorded at the Mavalane International Airport weather station located within a radius of 5 km from the trial site (Table 3.2). The mean of day length (Table 3.2), from the day of emergence to onset of flowering observed in 50% of the plants, was obtained using solar calculation of the National Oceanic and Atmospheric Administration (ESRL n.d.). The crops were harvested at 76, 70, 69, 78, 67 and 58 DAT for RS1, RS2, RS3, DS1, DS2 and DS3 respectively.

Table 3.2: Mean temperature, day length, total amount of water recorded (rainfall plus irrigation) and number of days with rainfall during the field trial periods

	Temperature — (°C) —	Day length (Hour day^{-1})	Treatment	Number of days with rainfall	Rainfall ———	Irrigation (mm)	Total ———
RS1	27.2	13.41	80AW	22	459.0	264.1	723.1
			50AW			148.3	607.3
			20AW			106.8	565.8
RS2	22.9	11.91	80AW	18	109.5	267.3	376.8
			50AW			185.6	295.1
			20AW			126.2	235.7
RS3	25.4	12.86	80AW	15	83.0	235.7	318.7
			50AW			161.1	244.1
			20AW			104.3	187.3
DS1	22.6	11.59	80AW	1	1.5	297.7	299.2
			50AW			181.4	182.9
			20AW			130.0	131.5
DS2	21.0	11.03	80AW	2	7.0	214.9	221.9
			50AW			151.4	158.4
			20AW			104.6	111.6
DS3	22.8	10.97	80AW	5	18.5	213.7	232.2
			50AW			161.4	179.9
			20AW			105.4	123.9

80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW; RS1, January-March 2014; RS2, September-November 2014; RS3, February-April 2015; DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015.

The treatments involved two species, *A. hybridus* and *A. tricolor*, and three levels of watering regimes, irrigated at 80% (80AW), 50% (50AW) and 20% (20AW) of total available water (TAW). The experimental design was a randomized complete block with a 3 x 2 factorial arrangement with six replications. The plots of 1.75 m^2 each were separated from each other by 1.5 m wide bare soil strips to prevent lateral flow and run-on of water treatments. Four plants from the center of each plot were randomly selected as experimental units in order to eliminate edge effects.

The water treatments started at 12 DAT. The soil water (SW) was monitored using a Time Domain Reflectometer (TDR) HydroSense II Water Sensor with 20 cm Rods (Campbell Scientific, Inc.) previously calibrated. The calibration was conducted using 65 samples of soil, representing different soil water status from saturation to very dry, collected at 0-40 cm depth in the trial site. For the calibration gravimetric soil moisture were determined by drying soil samples in the oven at 105 ^{o}C to a constant weight. A calibration curve was generated by regressing volumetric water content against the TDR value displayed.

The irrigation schedule was based on SW content. Since the root of *Amaranthus cruentus* reach depths of 13 and 35 cm in one and three months after planting respectively (Fasinmirin et al. 2008) and the plants have low capacity for geotropic growth after transplanting (Egel and Martyn 2008), the SW monitoring was conducted at 0-40 cm depth to cover the root zone where the most effective roots are located. From 12 to 30 DAT, the SW was measured every two days at 0-20 cm depth with a handheld TDR and the plants were irrigated when 20.0, 16.4 and 12.7 mm of SW was reached for 80AW, 50AW and 20AW treatments respectively; after 30 DAT soil water measurements were done at the top 40 cm and 41.4, 33.5 and 25.6 mm of SW were used as indicators to irrigate. At 20-40 cm depth, the SW was measured through a hole of 20 cm depth placed in the plots between the four central plants. A 16 cm diameter PVC tube was placed in the hole and was covered with a white plastic sheet to avoid water loss by evaporation. In order to guarantee that SW was not lower than the pre-defined levels of 80AW, 50AW and 20AW treatments, the amount of 7.4 mm of water were applied at irrigation intervals varying from one to two days, three to four and five to six days respectively. The trials were watered by hand using a watering can of 13 L capacity. Rainfall was recorded using the mean of 3 rain gauges distributed in the trial field. The total amount of water recorded during the trial periods for the different water level treatments is presented in Table 3.2.

Measurements

The initial plant height (H_0), from the soil level to the youngest fully expanded apex leaf, was measured 12 DAT using a tape measure. The final plant heights with inflorescence (H_{FI}), from soil surface to inflorescence apex, and without inflorescence (H_{FWI}), from soil surface to the bottom part of the inflorescence, were measured at harvesting. The height growth was calculated by the difference between H_{FI} and H_0. All other parameters were recorded at harvesting. The internode number (IN) was counted at the first node above the soil level to the beginning of inflorescence on the main stem. By dividing the H_{FWI} by IN the internode length was calculated. The leaf and side-shoot numbers were counted on the whole plant including secondary and tertiary shoots. The length and width of 14 randomly selected leaves of the 4 plants (experimental unit) were measured with a tape measure and the leaf area was calculated using a linear equation (Kvet and Marshall, 1971):

Leaf area (cm^2) = 0.654 x (L x W);

Where: 0.654 = leaf shape coefficient; L = length of leaf (cm); W = width of leaf (cm) measured halfway between the base and tip of the leaf.

The mean leaf area was used as leaf size and the total leaf area was calculated by multiplying leaf size with leaf number. The plants were divided into leaves, stems (main stem and side-shoots) and inflorescences, and dried at 80°C for 48 hours to determine the dry mass and aboveground biomass. Leaf calcium and nitrogen contents were analysed at the Soil Laboratory of the FAEF, using a composite dry leaf sample of the six trial replications per treatment as samples. The analysis was replicated during RS1, RS2, DS1 and DS2. The leaf calcium content was determined by the Complexometric Titration Method after digestion with nitric and perchloric acids (Waling et al. 1989) and the total nitrogen by Kjeldahl Method. The leaf crude protein (CP) content was calculated by multiplying N content with the factor 6.25.

Data analysis

The data collected from the six trials were subjected to analysis of variance using the MSTAT-C Version 1.2 computer program. Duncan´s New Multiple Range Test (DMRT) was used to compare treatment means. The analysis of variance was individually performed for each trial. To compare diverse climatic conditions and measure the response to treatments at different transplanting dates (TD) a combined analysis of variance over TD was performed with species, watering regime and season as factors for each type of season (rainy and dry).

Multiple linear regression analysis was performed using the SPSS Version 10.1.0 computer program. The relationships between mean air temperature and day length on leaf yield was analysed as described by Aiken and West (1991). This approach enhances interpretability of coefficients and reduces numerical instability for estimation associated with multicollinearity. The interaction term is defined by the product of the centered predictors. Centring the two predictors by subtracting their means eliminates correlations due to scaling of predictors and allows the analysis of the interaction.

Results and discussion

The rainfall and soil water (SW) measured in the top 40 cm of soil are presented in Figure 3.1. Measured SW exceeded the intended watering regimes (WR) during the rainy season due to the higher amount of recorded rainfall, and it was particularly evident during RS1. However, during the dry season, the measured SW values were around the intended WR guidelines.

Although interactions between WR and species (S) were significant for some parameters and seasons, the discussion will be focused on the main effects of WR and S given the general consistency in the results.

Vegetative growth

The height growth and internode length decreased with reduced total available water during all seasons (Table 3.3). However, the SW was higher than the predefined water level most of the time during RS1 (Figure 3.1). The decrease in height growth and internode length during RS1 might be explained by high sensitivity of cell expansion to water stress (Hsiao 1973), since water deficit reduces cell expansion which causes a decrease in stem growth. The decreasing plant height under low SW was also reported in *A. hybridus* (Masarirambi et al. 2012) and *A. tricolor* (Singh and Whitehead 1992). The reduction in internode length observed is also in agreement with results from other crops such as *Corchorus olitorius* (Shiwachi et al. 2008) and rice (Alizadeh et al. 2011).

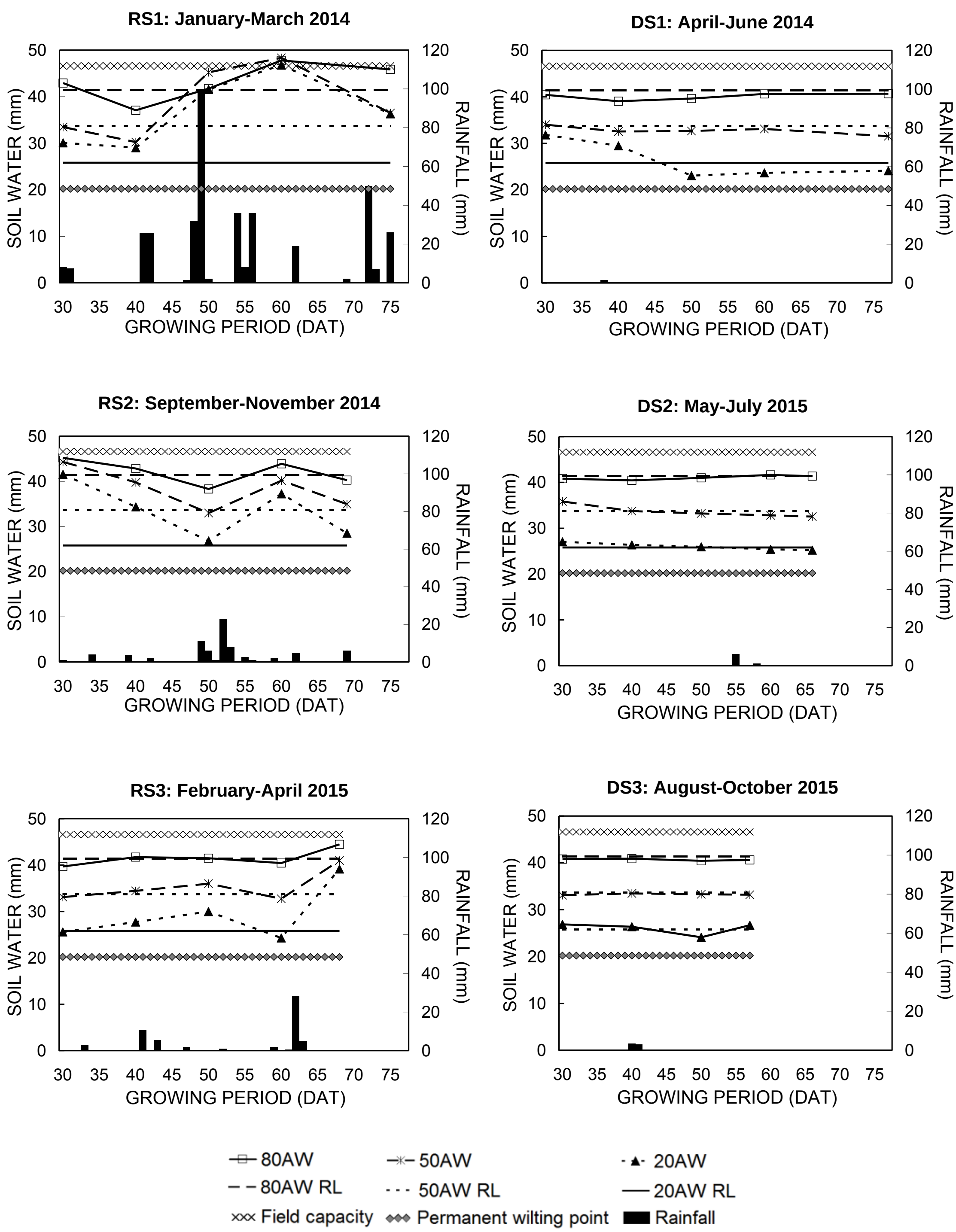

Figure 3.1: Rainfall and soil water (average of every 10 days) measured at the top 40 cm of soil during the different trial periods. The three required levels (RL) of watering regimes (80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW), field capacity and permanent wilting point are shown.

Table 3.3: Main effects of the watering regime and amaranths species on height growth, internode length, leaf and side-shoot number, and leaf area of amaranth leafy vegetables grown at the experimental station of Eduardo Mondlane University

Seasons		Treatment	Height growth	Internode length	Leaf	Side-shoot	Leaf size	Total leaf area
			(cm)		(number)		(cm^2)	(cm^2 $plant^{-1}$)
Rainy	RS1	80AW	164.30**a**	4.08**ab**	176.5	29.9	81.8	14297.9
		50AW	166.02**a**	4.21**a**	176.7	29.9	78.0	13998.7
		20AW	150.43**b**	3.82**b**	173.8	29.2	74.7	13137.7
			*	*	ns	ns	ns	ns
		A. hybridus	164.91	4.24	161.8	27.6	83.3	13582.6
		A. tricolor	155.59	3.82	189.6	31.8	73.0	14040.3
			ns	**	*	**	**	ns
		CV (%)	9.59	8.18	20.18	8.40	14.19	27.23
	RS2	80AW	70.43**a**	2.78**a**	94.0	15.5	28.9	2927.2
		50AW	65.01**b**	2.62**a**	97.9	15.6	26.3	2885.8
		20AW	57.46**c**	2.24**b**	100.8	14.8	24.3	2568.3
			**	*	ns	ns	ns	ns
		A. hybridus	65.96	2.71	91.3	14.6	29.5	2896.0
		A. tricolor	62.64	2.38	103.8	16.0	23.5	2691.6
			ns	*	*	**	*	ns
		CV (%)	15.03	18.53	14.46	6.70	27.39	37.09
	RS3	80AW	101.39**a**	3.27**a**	184.5	28.4	43.4	7976.8
		50AW	89.43**b**	3.14**ab**	183.5	27.5	41.0	7523.0
		20AW	80.19**c**	2.94**b**	171.1	26.3	38.7	6636.7
			**	*	ns	ns	ns	ns
		A. hybridus	94.33	3.28	164.2	26.0	43.0	7097.5
		A. tricolor	86.35	2.96	195.2	28.8	39.1	7660.1
			ns	**	**	**	*	ns
		CV (%)	14.44	8.85	9.95	7.39	11.46	17.70
Dry	DS1	80AW	63.05**a**	2.30**a**	108.9**a**	15.8**a**	30.5**a**	3332.0**a**
		50AW	62.93**a**	2.42**a**	102.1**b**	15.6**a**	27.4**b**	2786.7**b**
		20AW	48.92**b**	1.91**b**	79.8**c**	13.7**b**	19.1**c**	1759.8**c**
			**	**	**	**	**	**
		A. hybridus	60.50	2.32	89.7	14.5	30.0	2893.3
		A. tricolor	56.10	2.10	104.2	15.6	21.4	2359.1
			ns	*	*	*	**	ns
		CV (%)	13.47	13.85	20.52	10.45	29.47	31.94
	DS2	80AW	67.82**a**	2.70**a**	114.0**a**	18.7**a**	29.7**a**	3420.7**a**
		50AW	51.25**b**	2.13**b**	102.7**b**	18.7**a**	18.9**b**	1965.8**b**
		20AW	35.31**c**	1.73**c**	71.1**c**	15.9**b**	14.4**c**	1024.1**c**
			**	**	**	**	**	**
		A. hybridus	52.41	2.31	81.9	16.3	22.6	2009.0
		A. tricolor	50.51	2.06	110.0	19.3	19.4	2264.7
			ns	**	**	**	*	ns
		CV (%)	13.91	9.91	15.78	8.79	21.63	35.77
	DS3	80AW	57.11**a**	2.64**a**	95.6**a**	16.1**a**	21.7**a**	2084.2**a**
		50AW	41.11**b**	2.05**b**	81.6**b**	14.1**b**	18.0**b**	1494.7**b**
		20AW	30.83**c**	1.70**c**	63.5**c**	11.6**c**	14.9**c**	965.1**c**
			**	**	**	**	*	**
		A. hybridus	43.78	2.22	71.7	13.2	20.2	1505.8
		A. tricolor	42.24	2.04	88.9	14.6	16.2	1523.5
			ns	*	**	*	*	ns
		CV (%)	10.14	10.48	19.58	13.28	30.60	44.68

In each section, means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; * and **, represent significance at P<0.05 and P<0.01, respectively; CV, coefficient of variation; 80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW; RS1, January-March 2014; RS2, September-November 2014; RS3, February-April 2015; DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015.

The leaf number and leaf area decreased significantly with each decrease in SW from 80% to 20% of AW during dry seasons (Table 3.3). It is a typical morphological response to water deficit as a mechanism used by plants to reduce water loss (Anjum et al. 2011, Akinci and Lösel 2012). However, the side-shoot numbers decreased only at 20AW. This was probably related to the decrease in height growth below 50 cm at 20AW during the dry season (Table 3.3). Masarirambi et al. (2012) also found a decrease in leaf number and leaf area of *A. hybridus* with reduced irrigation levels from 85% to 40% of field capacity under greenhouse conditions. Singh and Whitehead (1992) reported different results, the leaf and branch numbers, and leaf area of *A. tricolor* were reduced only at wilting point under greenhouse conditions.

As shown in Table 3.3, it is clear that the drought effect was less pronounced during the rainy season due to rainfall (Figure 3.1) that prevented the maintenance of the predefined soil water deficits. This is evident since water availability was less restricted during the rainy season and thus low severity of water stress was experienced. The duration of water stress and the plant's ability to develop adaptive mechanisms to survive in adverse conditions, affect plant growth according to its severity (Akinci and Lösel 2012). According to Slabbert et al. (2004), *A. hybridus* and *A. tricolor* decreased leaf area to reduce leaf water loss under drought stress and recover quickly to restore leaf hydration if re-watered. Since the WR affected height growth and internode length but not the leaf number and leaf area during the rainy season, it appears that the crop was exposed to intermittent stress for short periods. This suggests that both species are well adapted as leafy vegetables to cope with short intermittent moisture stress periods during the rainy season.

Shorter internodes, more leaves and side-shoots and smaller leaves were developed by *A. tricolor* compared to *A. hybridus* (Table 3.3). Although *A. tricolor* produced more leaves, the total leaf area did not differ from that of *A. hybridus* as a result of smaller leaves developed by *A. tricolor*. The differences between the two species may be attributed to the difference in their genetic variation.

Aboveground yield components

During RS3 and the dry seasons, aboveground biomass decreased at 50AW and 20AW compared to 80AW (Table 3.4). This reduction followed the reduction in leaf, stem and inflorescence yield during the dry seasons. Similar results for *A. hybridus* are presented by Masarirambi et al. (2012), under greenhouse conditions; Singh and Whitehead (1992) only found a reduction in aboveground biomass of *A. tricolor* under severe water limitations. During the dry seasons, the reduction in aboveground biomass was consistent with reductions observed in leaf number and leaf area. However, during RS3, the leaf yield was not affected by WR. The inflorescence yield decreased at 50AW and 20AW except in RS1 (Table 3.4). This supports the statement by Blum (2011) that the flowering and reproduction stages of plants are the most susceptible to water deficit.

Results of the combined analysis over transplanting date (TD) show that the leaf yield was not significantly affected by WR during rainy seasons (Table 3.5). This indicate that the water deficit was not high enough to affect leaf yield in both species as the amount of rainfall received was high during the growing period (Figure 3.1).

During the dry season a significant interaction with regard to leaf yield was shown between TD and WR (Table 3.5). The leaf yield decreased at all TD with a decrease in SW from 80% to 20% of AW (Figure 3.2).

Table 3.4: Main effects of the watering regime and amaranths species on aboveground yield components of amaranth leafy vegetables grown at the experimental station of Eduardo Mondlane University

			Yield on dry mass basis			
Seasons		Treatment	Aboveground biomass	Leaf	Stems	Inflorescence
			(g plant^{-1})			
Rainy	RS1	80AW	109.36	25.33	44.51	39.52
		50AW	107.65	24.72	43.13	39.81
		20AW	99.97	24.86	39.12	35.99
			ns	ns	ns	ns
		A. hybridus	109.26	26.14	42.97	40.16
		A. tricolor	102.06	23.80	41.54	36.72
			ns	ns	ns	ns
		CV (%)	24.47	21.94	27.13	27.29
	RS2	80AW	37.03	6.51	8.00	22.52**a**
		50AW	34.18	5.88	7.60	20.72**b**
		20AW	27.07	5.28	6.23	15.52**c**
			ns	ns	ns	*
		A. hybridus	35.79	6.33	7.53	21.94
		A. tricolor	29.73	5.44	7.03	17.26
			ns	ns	ns	*
		CV (%)	33.01	27.49	42.82	34.10
	RS3	80AW	68.68**a**	16.01	21.53**a**	31.13**a**
		50AW	59.29**b**	15.60	16.77**b**	26.93**b**
		20AW	51.01**c**	13.52	13.96**c**	23.52**c**
			**	ns	*	**
		A. hybridus	58.27	15.26	16.29	26.71
		A. tricolor	61.05	14.82	18.54	27.68
			ns	ns	ns	ns
		CV (%)	21.63	20.05	34.49	19.02
Dry	DS1	80AW	30.42**a**	6.49**a**	6.65**a**	17.28**a**
		50AW	28.48**b**	5.91**b**	6.56**a**	15.99**b**
		20AW	19.29**c**	3.88**c**	4.52**b**	10.89**c**
			**	**	**	**
		A. hybridus	27.62	5.67	6.21	15.73
		A. tricolor	24.50	5.19	5.61	13.71
			ns	ns	ns	ns
		CV (%)	25.72	23.62	23.19	30.01
	DS2	80AW	28.27**a**	8.04**a**	7.28**a**	12.98**a**
		50AW	16.88**b**	4.64**b**	4.50**b**	7.77**b**
		20AW	8.08**c**	2.54**c**	1.98**c**	3.56**c**
			**	**	**	**
		A. hybridus	17.55	4.76	4.36	8.46
		A. tricolor	17.93	5.39	4.81	7.75
			ns	ns	ns	ns
		CV (%)	25.13	27.38	7.28**a**	25.75
	DS3	80AW	28.19**a**	5.48**a**	7.08**a**	15.67**a**
		50AW	13.64**b**	3.17**b**	3.34**b**	7.13**b**
		20AW	9.52**c**	2.00**c**	1.83**c**	5.67**c**
			**	**	**	**
		A. hybridus	16.72	3.57	3.98	9.17
		A. tricolor	17.52	3.53	4.18	9.81
			ns	ns	ns	ns
		CV (%)	21.28	37.54	25.21	26.40

In each section, means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; * and **, represent significance at *P*<0.05 and *P*<0.01, respectively; CV, coefficient of variation; 80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW; RS1, January-March 2014; RS2, September-November 2014; RS3, February-April 2015; DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015.

Except under 80AW, the leaf yield generally was higher in DS1 than in DS2 which in turn was higher than DS3 (Figure 3.2). The temperature recorded in DS2 was lower than in DS3 (Table 3.2). This indicates that the increased leaf yield was not due to increased temperatures. However, the longer day length and higher leaf number observed in DS2 compared to DS3 (Tables 3.2 and 3.3) may indicate that the leaf yield was greatly affected by day length. Since short days affects the onset of flowering and the metabolic sink benefits developing inflorescences (Hopkins 1999), the low leaf yield in DS3 was due to a lower leaf number caused by short day lengths. A significant reduction in *A. tricolor* vegetative growth with a decrease in day length and temperature was also found by Whitehead et al. (2002).

Table 3.5: Summary of combined analysis of variance over transplanting date on leaf yield during the rainy and dry seasons

Source	df	RAINY SEASON		DRY SEASON	
		Probability	Leaf yield (g plant^{-1})	Probability	Leaf yield (g plant^{-1})
Transplanting date (TD)	2	0.0000**		0.0000**	
January RS1			24.97**a**		
September RS2			5.89**c**		
February RS3			15.04**b**		
Repetitions within TD	15	0.0000**		0.0000**	
Watering regime (WR)	2	0.2831^{ns}		0.0000**	
TD x WR	4	ns		0.0011**	(#)
Species (S)	1	0.0927^{ns}		ns	
TD x S	2	ns		0.2058^{ns}	
WR x S	2	ns		ns	
TD x WR x S	4	ns		0.2570^{ns}	
Error	75				
Total	107	CV = 24.38%		CV = 28.39%	

(#) Mean comparison are presented in Figure 3.2.
In each section, means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; **, represent significance at P<0.01; CV, Coefficient of variation; RS1, January-March 2014; RS2, September-November 2014; RS3, February-April 2015.

During the rainy seasons the leaf yield significantly increased from 5.89 g plant^{-1} in RS2 to 24.97 g plant^{-1} in RS1 (Table 3.5). This yield increase followed the increase in temperature and day length recorded during RS1 (Table 3.2), indicating that these two climatic factors greatly affected leaf yield during the rainy seasons. However, there was no significant difference between the species and this was consistent over all TD (Table 3.5), indicating that both species are similarly adapted to the different climate conditions during the rainy and dry seasons.

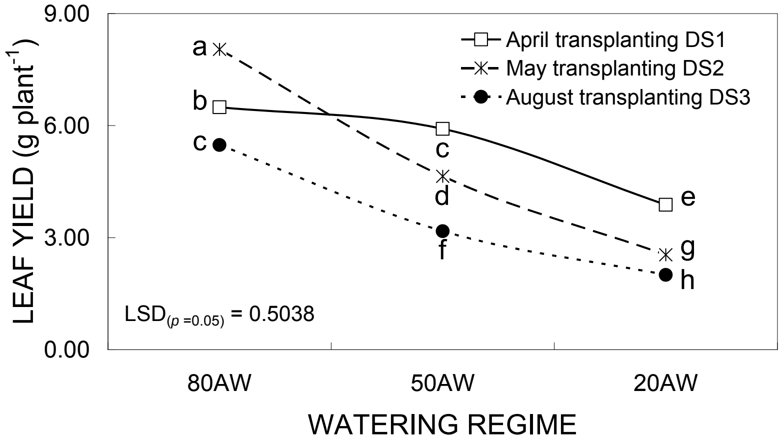

Figure 3.2: Interaction between the watering regime (80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW) and transplanting date affecting the leaf yield of amaranths during dry seasons (DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015). Means followed by the same letters are not significantly different at 5% level probability (DMRT).

The highest leaf yields, between 15 and 25 g plant^{-1}, were achieved during the rainy season when the transplanting occurred in January and February which is the period of the year with long and warm days. In relation to low leaf yields during the dry season, both species produced between 6 and 8 g plant^{-1} when the transplanting were made in April and May with irrigation (80AW condition).

Relationships between temperature and day length in terms of leaf yield

Results of the relationship between temperature and day length using the centered predictor variables (Table 3.6A) showed that the significant interaction effect accounted for 9.1% of variance in leaf yield as revealed by F-change and coefficient of determination (R-square) change in Table 3.6B.

Table 3.6: Multiple linear regression of two continuous variables, temperature (T) and day length (DL) on amaranth leaf yield (LY) in different seasons; **A.** Mean and standard deviation; **B.** Analysis of regression without and with interaction term; **C.** Regression coefficients

A. Mean and standard deviation (*N* of case = 12)

	Mean		Standard deviation	
	Uncentered	Centered	Uncentered	Centered
LY	9.99		8.03	
T	23.65	0	2.14	2.14
DL	11.96	0	0.94	0.94
T x DL		1.74		1.79

B. Analysis of regression without and with interaction term

Interaction term	R-square	Change statistics		Significance (Sig.)
		R-square change	Sig. F change	
Without	0.904	0.904	0.000	0.000
With	0.995	0.091	0.000	0.000

C. Regression coefficients

Y	Intercept	$T_{Centered}$	$DL_{Centered}$	$T_{Centered}$ x $DL_{Centered}$
LY	6.956**	0.901*	4.082**	1.744**

* and ** represent significance at *P*<0.05 and *P*<0.001, respectively.

The proposed model was highly significant and successfully accounted for 99.5% of the total variation in leaf yield expressed as R-square (Table 3.6B). The significant and positive weight of the interaction coefficient (Table 3.6C) indicates that the effect of temperature on leaf yield is greater for higher values of day length and less for smaller values of day length. For every 1°C increase in the temperature an increase in leaf yield of 2.54 g plant^{-1} is expected under a constant day length of 12.9 hours day^{-1} during the rainy season while under a constant 11.02 hours day^{-1} a decrease in leaf yield of 0.74 g plant^{-1} is expected during the dry season as revealed by the proposed centered models (Figure 3.3). It is clear that the effect of temperature on the leaf yield is greatly determined by the length of day for both species.

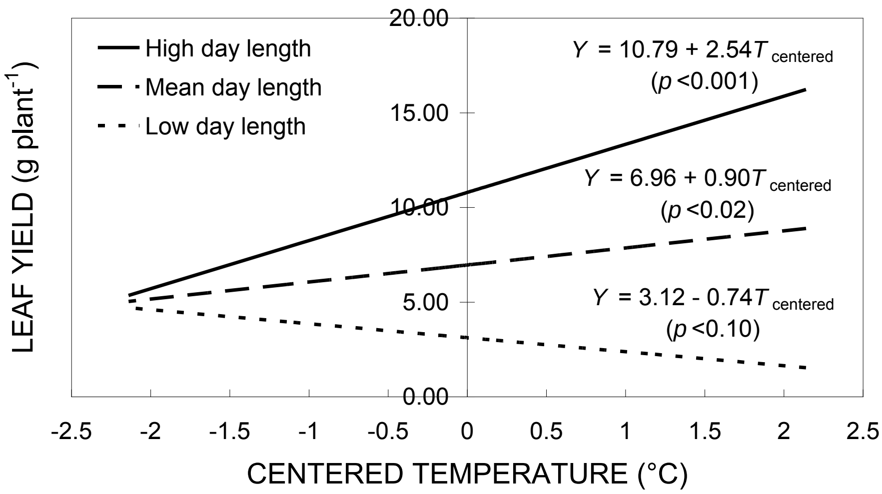

Figure 3.3: Regression of leaf yield on temperature (T) at three levels of day length (DL): low DL, one standard deviation below the mean of DL; mean DL, at the mean of DL; and high DL, one standard deviation above the mean of DL.

As the temperature is linked to the process of photosynthesis and these two species are C_4 plants, the high leaf yield is expected at high temperature and day length. It is noteworthy here that the peak of photosynthetic rate was found in *A. tricolor* at 35 ºC (Lin and Ehleringer 1983).

These results provide useful information which could be beneficial for amaranth production in terms of appropriate transplanting date, according to climatic conditions, in southern Mozambique. In this region, the monthly mean of the day length, from September to December, increase from 11.95 to 13.66 hours day^{-1} and then gradually decrease to 10.90 hours in May. Using this information and the results from TD, both species can be cultivated throughout the year and a variation in leaf yield from 6 to 25 g plant^{-1} might be obtained, if transplanting from June to August is avoided.

Calcium and crude protein contents in the leaves

A significant interaction with regard to leaf calcium content was shown between WR and species (Figure 3.4A). The leaf calcium content increased at 50AW and 20AW compared to 80AW in both species and the highest values of the 4726 and 5675 mg 100 g^{-1} were found in *A. tricolor* at 50AW and 20AW respectively. The increased leaf calcium content at low water levels could be attributed to the fact that with increased water stress some plants develop a selective uptake for specific elements, Ca^{2+} being one of them (Akinci and Lösel 2012). Note that the calcium is involved in regulating the plant response to drought condition and an increase of intracellular calcium is expected (Bowler and Fluhr 2000). However, Luoh et al. (2014) found no significant difference in leaf calcium content of *A. cruentus* and *A. hypochondriacus* under water-deficient levels in greenhouse conditions. The difference in drought effects on leaf calcium content in this study was probably due to different experimental conditions and species used.

The leaf crude protein (CP) content in both species was high at 50AW and 20AW conditions (Figure 3.4B). This might be explained by an increase in specific proteins synthesized in leaves as osmoprotectants accumulate for osmotic adjustment in response to water stress (Akinci and Lösel 2012).

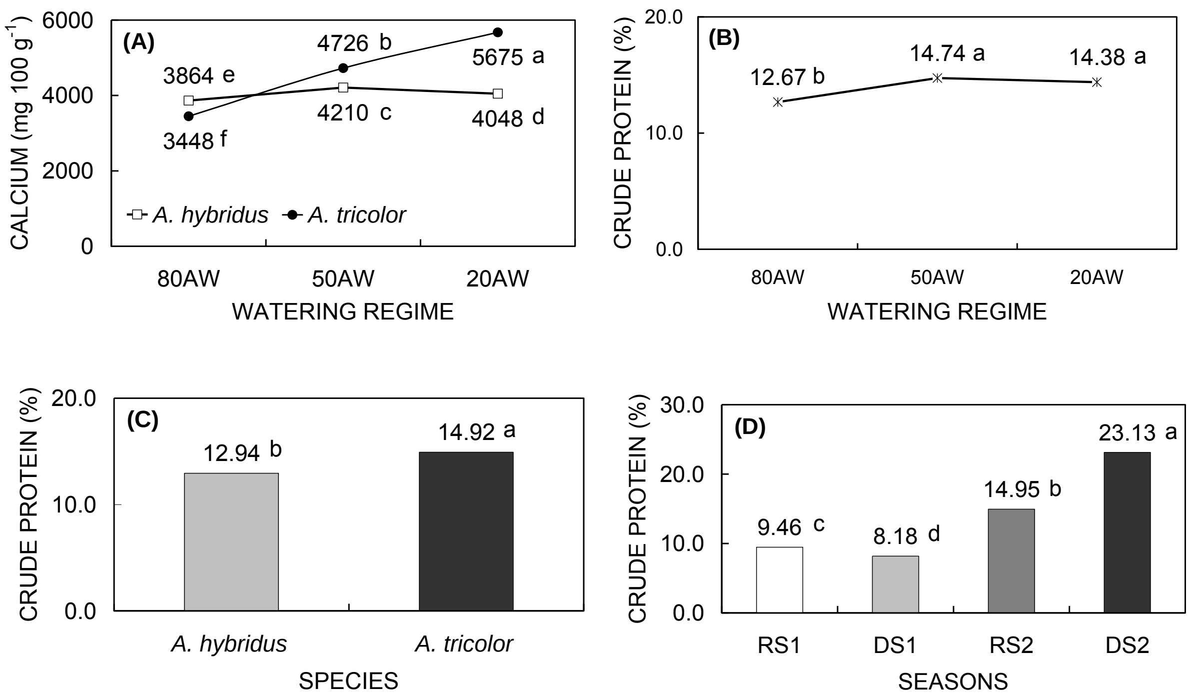

Figure 3.4: (**A**) Leaf calcium content affected by an interaction between species and watering regimes (80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW), and effects of: (**B**) watering regimes, (**C**) species and (**D**) seasons (RS1, January-March 2014; DS1, April-June 2014; RS2, September-November 2014; DS2, May-July 2015) on crude protein content in leaves of *A. hybridus* and *A. tricolor*. In each figure, means followed by the same letters are not significantly different at 5% level probability (DMRT).

The highest value of 14.92% CP was obtained in *A. tricolor* leaves (Figure 3.4C) and was slightly lower compared to 17.92% in *A. hybridus* (Akubugwo et al. 2007) and 15-16% in *A. hybridus* and *A. tricolor* under hot conditions (Modi 2007). Nitrogen fertilization positively affects the leaf CP content of *A. hypochondriacus* (Abbasi et al. 2012). The fact that only 150 kg ha^{-1} of urea were applied during RS1 and DS1, and

225 kg ha^{-1} applied during RS2 and DS2, the low CP content observed in RS1 and DS1 (Figure 3.4D) was probably due to low nitrogen availability.

Conclusions

The vegetative growth of *A. hybridus* and *A. tricolor* decreased with the decrease of soil water from 80% to 20% of total available water. However, the leaf yield was less susceptible to intermittent drought stress that occurred in intervals of sporadic rainfall during the rainy season. Both species had similar behavior in relation to different soil water and climate conditions during the rainy and dry seasons in southern Mozambique.

The calcium and crude protein content in the leaves were improved at lower levels of soil water, 50% and 20% of available water. The highest leaf calcium and crude protein content were obtained in *A. tricolor*.

Temperature greatly affected the maximum leaf yield of both species during the rainy season with increased day lengths, while the low yield was determined by the length of the day during the dry season under short day lengths.

This study revealed that the *A. hybridus* and *A. tricolor*, as nutritive leafy vegetables, have the potential to produce a sustainable crop throughout the year. During the rainy season both species showed potential to be cultivated under rain-fed condition with supplemental irrigation. However, during the dry season, they might be cultivated as an irrigated crop with less than 300 mm of water required.

Acknowledgement - The authors are grateful to the SHARE Project and the National Research Fund of Mozambique (FNI) for financial support.

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Chapter 4

Effect of watering regimes in different seasons on grain yield and nutrient contents of *Amaranthus hybridus* and *A. tricolor* in southern Mozambique

Abstract

The high nutritional value of amaranth grain makes it attractive for the diet of rural people living in dry semi-arid areas of southern Mozambique. From December 2013 to October 2015, a study was implemented with *Amaranthus hybridus* and *A. tricolor* aiming to assess: (a) the effect of different watering regimes on flowering, grain yield and grain crude protein content of the two species during the rainy and dry seasons, and (b) the effect of day length and minimum temperature on the time to flowering. Six field trials were conducted in Maputo in a randomized complete block design with 2x3 factorial layout with six replications. This study revealed that panicle and internode lengths as well as straw and grain yields of both species decrease when the soil water content decrease. Conversely, the time to flowering and the grain crude protein contents were unaffected by the water deficit. The onset of flowering was significantly delayed by long days. Regarding grain yield and harvest index, *A. tricolor* showed better performance under day lengths above 12 hours day^{-1}. When irrigation was applied to ensure soil water content at 80% of total available water, both species produced all year long.

Keywords: amaranth, crude protein, grain yield, harvest index, watering regime

Introduction

Amaranth is a multi-purpose plant used as a leafy vegetable or as a grain crop for human diet in different parts of the world. As a grain crop, it is part of the so-called pseudocereals and possesses more nutritional benefits than cereals. It contains high quality proteins, particularly the amino-acid lysine (Pospišil et al. 2006, Venskutonis and Kraujalis 2013). Due to its nutritional value, amaranth might contribute to food security and dietary diversification in southern Mozambique if crop production practices are sustained over time and are ecologically adapted to arid and semi-arid conditions.

Amaranth, as grain crop, can be produced in arid and semi-arid regions (Schahbazian et al. 2006), however some studies have revealed a reduction in grain yield under water deficit (Mng'omba et al. 2003, Olufolaji et al. 2010, Mlakar et al. 2012). Some amaranth species are considered short day plants; they are induced to flowering when the day length is less than 12 hours (Ebert et al. 2011). Though, flowering can be delayed with high night temperatures above 22 ºC in short day plants (Erwin 2005).

The south of Mozambique is characterized to have two distinct seasons: the rainy and hot season and the dry and cool season. This region is regularly exposed to drought which has been the main climatic factor that limits food production in dry and semi-arid areas. In these areas, the mean annual temperature range from 23 to 26 ºC and the average annual rainfall varies from 500-600 mm inland and 350 mm in the driest interior. In terms of day length, the longest day, 13.69 hours day^{-1}, occurs during the rainy season in December, and the shortest, 10.58 hours day^{-1}, in the dry season in June. Rain-fed agricultural production is the farming system prevalent in the region with the major crops being maize, cassava and beans. Although the production of these food crops have slightly increased over the last two decades, the diet diversification is still inadequate for about 50% of households in the southern Provinces of Mozambique (RM 2010).

Amaranthus hybridus and *A. tricolor*, as leafy vegetables, showed to be relatively tolerant to moisture deficit, however, during the flowering stage, they were very sensitive to water deficits that occurred in short intervals of no watering throughout the rainy season in southern Mozambique (Chapter 3). Drought stress during the grain filling stage reduced grain yield in *A. tricolor* (Mng'omba et al. 2003). The grain yields of *A. hybridus* and *A. tricolor* were also reduced when the temperature dropped from 27/21 °C day/night to 21/15 °C (Modi 2007). The stimulus of the day length on grain development and maturity plays a significant role in plants that are sensitive to photoperiod. *Amaranthus tricolor* is a quantitative short day plant (Grubben 2004) and *A. hybridus* a day neutral plant meaning the day length does not affect flowering (Mattson and Erwin 2005).

The grain yield is strongly related to sink strength being the product of sink size and sink activity (Hopkins 1999), the allocation of biomass to filling the grain (Hopkins 1999, Fageria et al. 2006) and the growth stage and severity that the plants are exposed to water stress (Blum 2011). Since the day length induces flowering in photoperiod-sensitive plants and the grain development becomes the dominant sink (Hopkins 1999), time to flowering and the harvest index can thus be of major importance to assess the growing conditions and identify constraints to the grain yield, even more relevant than lack of water.

There is no information relating to *A. hybridus* and *A. tricolor* grain production in Mozambique. Understanding the constraints and opportunities for developing a sustainable production plan for these nutritive grain crops may contribute to improve food security and diet diversification. Hence, a study was conducted under field conditions to explore the vegetative (Chapter 3) and reproductive phases of these two species. The present study focus on the reproductive aspect, the grain, aiming to evaluate: (a) the effect of watering regimes on flowering, grain yield and grain crude protein content of *A. hybridus* and *A. tricolor* during the rainy and dry seasons in southern Mozambique; (b) the effect of day length and minimum night temperature on the time to flowering for both species. The experimental data used in this article were collected from the research study reported in Chapter 3.

Material and methods

A factorial experiment (2 x 3) with two species (*A. hybridus* and *A. tricolor*) and three levels of watering regimes (i. irrigated at 80% of total available water [80AW], ii. irrigated at 50% of total available water [50AW] and iii. irrigated at 20% of total available water [20AW]) was conducted at the experimental station of the Faculty of Agronomy and Forestry Engineering (FAEF), Eduardo Mondlane University (UEM) in Maputo (25°57′ S, 32°34′ E; 60 m above sea level) during six seasons. Three were conducted during the rainy seasons: 15 January to 30 March 2014 (RS1), 23 September to 30 November 2014 (RS2), and 18 February to 27 April 2015 (RS3), and the other three during the dry seasons: 14 April to 30 June 2014 (DS1), 14 May to 20 July 2015 (DS2), and 11 August to 8 October 2015 (DS3).

The water treatments were applied from 12 days after transplanting (DAT) to grain harvesting time and the soil water (SW) was monitored using a Time Domain Reflectometer (TDR) HydroSense II Water Sensor with 20 cm Rods (Campbell

Scientific, Inc.) previously calibrated. The calibration was done in the trial site as described in Chapter 3. In order to monitor the total available water (TAW), a soil depth of 40 cm was selected to cover the zone where most effective roots are located. This was based on the finding of *A. cruentus* root depth (Fasinmirin et al. 2008) and the low capacity of geotropic growth in plants after transplanting (Egel and Martyn 2008). The SW content between 20 cm and 40 cm depth was measured as reported in Chapter 3. The water application frequency was determined by the SW content measured every two days using a handheld TDR. From 12 to 30 DAT, the plants were irrigated when 20.0, 16.4 and 12.7 mm of SW measured at 0-20 cm depth was reached in 80AW, 50AW and 20AW treatments respectively; after 30 DAT, 41.4, 33.5 and 25.6 mm of SW at the top 40 cm of soil were used as guide to irrigate. The amount of 7.4 mm of water per application was applied at irrigation intervals varying from one to two days, three to four and five to six days in 80AW, 50AW and 20AW treatments respectively to guarantee the SW was not below the pre-defined levels. The plants were irrigated by hand using a watering can of 13 L capacity. Three rain gauges spread in field trials were used to record the rainfall.

The experiments were arranged in a randomized complete block design and replicated six times. Each plot of 1.75 m^2 (1.40 x 1.25 m) consisted of twenty plants spaced at 25 cm in row and 35 cm between rows. The plots were separated by 1.5 m in order to prevent water contamination from one plot to another. The experimental unit was four inner plants of each plot randomly selected in order to eliminate the edge effect.

Seeds of *A. hybridus* and *A. tricolor* were sown on 17 December 2013, 18 August 2014, 20 January 2015, 18 March 2014, 13 April 2015 and 3 July 2015, for RS1, RS2, RS3, DS1, DS2 and DS3 respectively. The seedlings were germinated and grown in the nursery as described in Chapter 3. The soil in the trial site is a sandy soil with more than 91% sand, non-saline with less than 0.1 mS cm^{-1} of electrical conductivity and soil organic matter of about 0.48% in the top 20 cm decreasing to 0.17% at 20-40 cm depth. The permanent wilting point, field capacity and available water are 20.2, 46.6 and 26.4 mm respectively, in the top 40 cm. The fertilizer management and weather conditions (rainfall and mean temperature are shown in Figure 4.1) were as reported in Chapter 3.

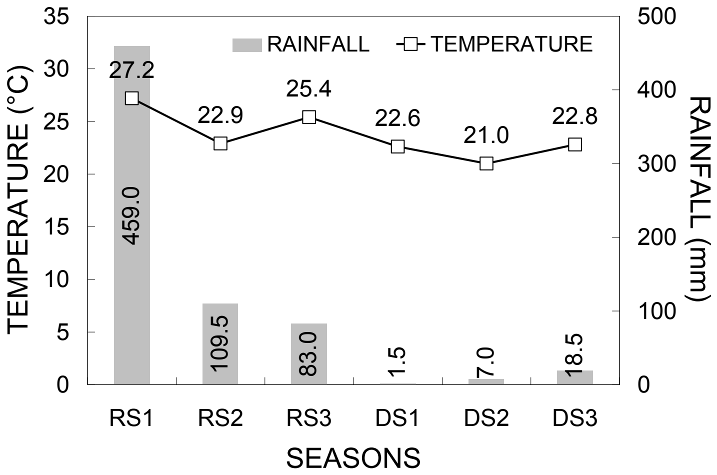

Figure 4.1: Rainfall and mean temperature during January-March 2014 (RS1), September-November 2014 (RS2), February-April 2015 (RS3), April-June 2014 (DS1), May-July 2015 (DS2), August-October 2015 (DS3).

The average of minimum temperatures recorded at the Mavalane International Airport weather station located within a radius of 5 km from the site trials, from the day of emergence to onset of flowering, was also calculated.

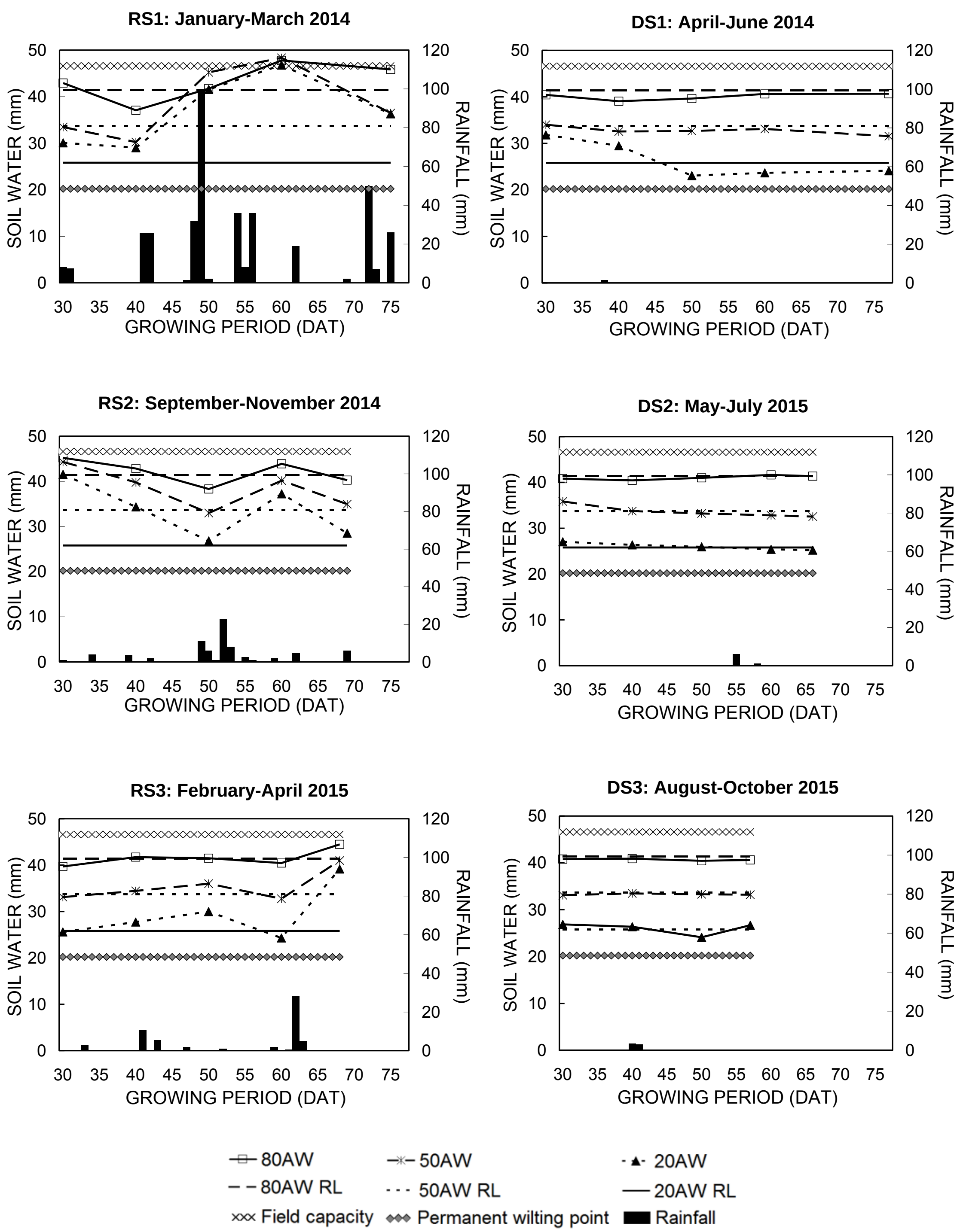

Figure 4.2: Rainfall and soil water (average of every 10 days) measured at the top 40 cm of soil during the different trial periods. The three required levels (RL) of watering regimes (80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW), field capacity and permanent wilting point are shown (Source: Chapter 3).

For the same period, the means of day lengths was obtained using solar calculations of the National Oceanic and Atmospheric Administration (ESRL n.d.). After 30 DAT, during the rainy and dry seasons, the plants grew with the average of SW as reported in Chapter 3 (Figure 4.2).

Measurements

Plants were monitored on a weekly basis and the timing of the first appearance of the inflorescence on the main stem was recorded. The time to flowering was recorded from the day on which seedlings emerged to the day where 50% of plants of the same plot displayed inflorescence primordia, and was expressed in number of weeks after emergence (WAE). The period of reproductive growth, from the time to flowering to grain maturity, was recorded in WAE. The panicle length (PL) of the main stem was measured at harvesting time from the first node of the panicle to the apex of the same panicle using a tape measure. The internode number on each panicle (INP) was counted from the first node of the panicle to the apex of the panicle. By dividing the PL by INP, the panicle internode length was calculated. The aboveground plant was harvested by cutting at soil level and dried at 70°C for 72 hours. After drying, the main panicle and other axillary panicles were separated from the plant. All panicles were divided into straw and grain in order to determine their dry mass per plant. The harvest index was calculated by dividing the grain dry mass by the aboveground biomass. Grain nitrogen contents were analysed at the Soil Laboratory of the FAEF, using a composite dry grain sample of the six trial replications per treatment as samples. The analysis was replicated during RS1, RS2, DS1 and DS2. The total grain nitrogen content was determined by the Kjeldahl

Method and the grain crude protein (CP) content was calculated by multiplying nitrogen content with 6.25.

Data analysis

Analyses of variances were conducted using the MSTAT-C Version 1.2 computer program. Duncan´s New Multiple Range Test (DMRT) was used to compare treatment means. The analysis of variance was individually performed for each trial. To compare diverse climatic conditions and measure the response to treatments at different sowing date (SD) a combined analysis of variance over SD was performed with species, watering regime and season as factors for each type of season (rainy and dry).

Multiple linear regression analysis was performed using the SPSS Version 10.1.0 computer program. The relationship between minimum air temperature and day length on the time to flowering was analyzed using the centered predictor variables as described by Aiken and West (1991). The centered predictor variables enhance interpretability of coefficients and reduce numerical instability for estimation associated with multicollinearity. The interaction term which is the product of the centered temperature and day length was also analyzed. The level of significance used for variables to enter into the regression equation was 20%. According to Gomez and Gomez (1984), the level of significance commonly used is between 10 and 20% allowing thus a larger number of variables to have the chance of entering into the regression equation.

Results and discussion

The interaction between watering regime (WR) and species (S) were significant for some parameters and seasons, however, the discussion will be focused on the main effects of WR and S given the consistency in the results.

Time to flowering

During all seasons, neither WR nor S main effects, nor their interactions, had a significant effect on time to flowering. The onset of flowering for a quantitative short day plant is basically determined by the day length. The day lengths during the rainy season were higher than during the dry season at the trial site (Figure 4.3A). The variation of the time to flowering observed was 7 to 10 WAE during the rainy season, and 6 to 7 WAE during the dry season, according to the vegetative growth period (Figure 4.3B). There was a delay of 3 and 1 weeks to start the flowering period for plants sown in December and January respectively (Figure 4.3B). Equal number of weeks to flowering (7 WAE) were found for plants sown in March, April and August. However, those sowed in July took 6 WAE to flower. This early flowering is probably due to the plants being exposed to day lengths of less than 12 hours (h) during the cropping season (Figure 4.3A).

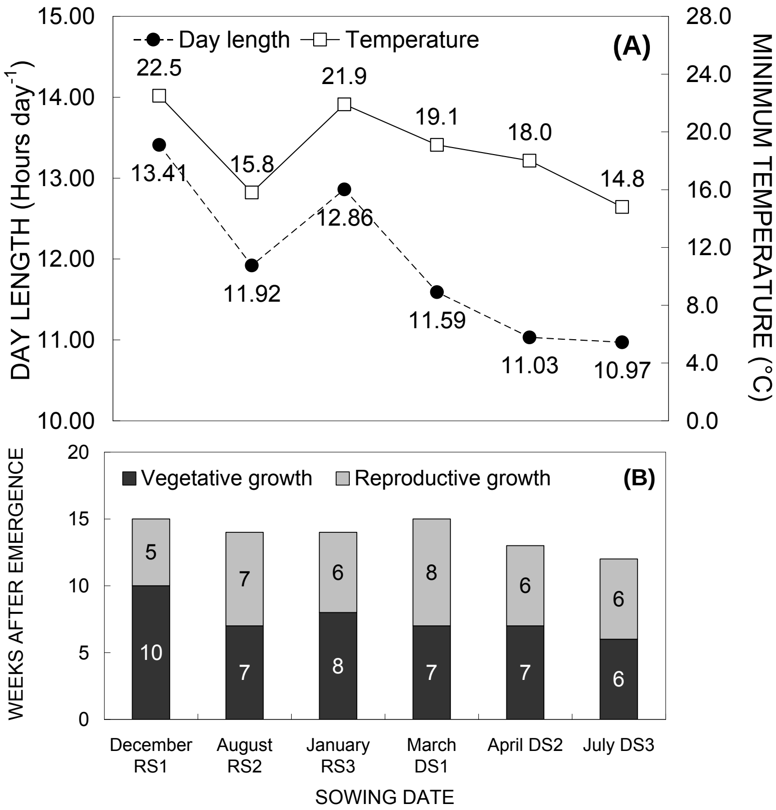

Figure 4.3: Day length and minimum temperature (**A**), vegetative and reproductive growth (**B**) during January-March 2014 (RS1), September-November 2014 (RS2), February-April 2015 (RS3), April-June 2014 (DS1), May-July 2015 (DS2), August-October 2015 (DS3).

Similar results were found by Huang et al. (2000) where *A. retroflexus*, quantitative short-day specie, took longer to reach reproductive stage when exposed to 14 and 16 h photoperiods compared to 8, 10 and 12 h. Comparing two seasons of long day length (> 12 h), RS1 (10 WAE) and RS3 (8 WAE), the two weeks delay of the flowering observed during RS1 appears to be associated to the slightly higher minimum daily temperature recorded in the season (Figure 4.3A). According to Erwin 2005, the high night temperature (> 22 °C) may delay flowering in short day plants.

Results of the relationships between day length and minimum daily temperature, using the centered predictor variables (Table 4.1A), showed a very low significant interaction effect on time to flowering as revealed by F-change (*p*=0.374) and coefficient of determination change (R-square=0.013) as presented in the Table 4.1B.

Table 4.1: Multiple linear regression of two continuous variables, minimum temperature (Tmin) and day length (DL) on time to flowering (TF); **A.** Mean and standard deviation; **B.** Analysis of regression including Tmin and interaction terms; **C.** Regression coefficients

A. Mean and standard deviation (*N* of case = 12)

	Mean		Standard deviation	
	Uncentered	Centered (C)	Uncentered	Centered (C)
TF	7.50		1.31	
DL	11.96	0	0.94	0.94
Tmin	18.68	0	2.98	2.98
DL x Tmin		2.14		2.20

B. Analysis of regression including Tmin and interaction terms

Term	R-square	Change statistics		Significance (Sig.)
		R-square change	Sig. F change	
DL_c	0.834	0.834	0.000	0.000
$Tmin_c$	0.867	0.033	0.170	0.000
DL_c x $Tmin_c$	0.881	0.013	0.374	0.000

C. Regression coefficients

Y	Intercept	Unstandardized coefficients		Standardized coefficients	
		DL_c	$Tmin_c$	DL_c	$Tmin_c$
TF	7.500***	0.895**	0.144*	0.642	0.326

*, ** and *** represent significance at *P*≤0.20, *P*≤0.05 and *P*≤0.001, respectively.

The proposed model, including minimum temperature term (F-change=0.170), was highly significant and successfully accounted for 86.7% of the total variation in time to flowering (Table 4.1B and C). Day length and minimum temperature significantly and positively contributed to onset of flowering in both species, indicating that a relative longer day length or higher minimum temperature result in flowering delay. As revealed by the standardized regression coefficients (Table

4.1C), the contribution of day length (0.642) to onset of flowering in both species was higher than that of minimum temperature (0.326). This indicates that the response of the plant in terms of flowering was more sensitive to variations in day length. Under a day length of 11.96 hours day^{-1} and a constant minimum temperature of 18.68 °C, the time to flowering is expected to be 7.5 WAE for both species, 99% confidence, as revealed by the intercept value of the proposed centered model (Table 4.1C). Under a constant day length of 11.96 hours day^{-1}, the increase in time to flowering is expected to be 0.144 WAE (1 day) for every 1°C increase in the minimum temperature. Since the monthly average of the day length and minimum temperature are higher during the rainy season in relation to the dry season, the flowering is expected to be later and earlier during rainy and dry seasons respectively. Although *A. hybridus* has been reported to be a day neutral plant (Mattson and Erwin 2005), in the experimental conditions of this study it has responded to the photoperiod in a similar way than *A. tricolor*.

Panicle length and internode length

During all seasons, the panicle and internode lengths were significantly affected by WR. The panicle length decreased at 50AW and 20AW while the internode length was significantly decreased at 20AW compared to 80AW treatments (Table 4.2). This reduction in panicle length as well as internode length at low water levels was probably due to the reduced cell division and cell enlargement under water deficit conditions (Farooq et al. 2009, Blum 2011, Akinci and Lösel 2012).

Table 4.2: Effects of the watering regime and amaranth species on panicle length, internode length in panicle, panicle straw mass, grain yield and harvest index

Seasons		Treatment	Panicle		Dry mass basis		
			Length	Internodes length	Panicle straw	Grain yield	Harvest index
			(cm)		(g plant^{-1})		(%)
Rainy	RS1	80AW	43.67**a**	1.87**a**	27.91	11.61	10.80
		50AW	41.86**b**	1.83**a**	29.41	10.40	9.87
		20AW	38.88**c**	1.57**b**	26.16	9.83	10.18
			*	*	ns	ns	ns
		A. hybridus	43.13	1.86	30.74	9.42	8.81
		A. tricolor	39.80	1.66	24.91	11.81	11.75
			*	*	*	*	**
		CV (%)	10.69	15.30	26.95	32.13	16.14
	RS2	80AW	35.28**a**	2.12**a**	12.13	10.38**a**	29.71**a**
		50AW	32.73**b**	1.88**b**	11.32	9.41**b**	29.58**a**
		20AW	31.54**c**	1.81**b**	8.93	6.62**c**	26.08**b**
			*	*	ns	**	*
		A. hybridus	34.99	2.16	12.40	9.53	28.07
		A. tricolor	31.38	1.71	9.19	8.07	28.83
			**	**	*	ns	ns
		CV (%)	10.47	14.56	38.86	29.68	11.63
	RS3	80AW	31.73**a**	1.38**a**	16.67**a**	14.46**a**	21.39
		50AW	29.78**b**	1.31**ab**	13.37**b**	13.57**b**	23.01
		20AW	27.31**c**	1.20**b**	11.93**c**	11.59**c**	22.89
			**	*	**	*	ns
		A. hybridus	30.73	1.36	14.68	12.03	20.68
		A. tricolor	28.48	1.23	13.29	14.38	24.18
			*	**	ns	*	**
		CV (%)	10.60	10.61	20.64	20.72	12.22
Dry	DS1	80AW	33.68**a**	2.02**a**	13.80**a**	3.48**a**	11.97
		50AW	31.86**b**	1.86**ab**	12.63**b**	3.37**a**	12.24
		20AW	26.45**c**	1.66**b**	8.72**c**	2.18**b**	12.08
			**	*	**	*	ns
		A. hybridus	32.27	1.99	12.78	2.96	11.09
		A. tricolor	29.06	1.70	10.65	3.06	13.10
			*	*	ns	ns	*
		CV (%)	11.90	17.70	30.14	36.47	18.59
	DS2	80AW	27.53**a**	1.99**a**	9.55**a**	3.42**a**	12.14**b**
		50AW	21.07**b**	1.65**b**	5.33**b**	2.41**b**	14.67**a**
		20AW	17.13**c**	1.50**b**	2.39**c**	1.18**c**	14.33**a**
			**	**	**	**	*
		A. hybridus	22.94	1.81	5.88	2.57	15.08
		A. tricolor	20.87	1.62	5.63	2.10	12.35
			*	*	ns	ns	**
		CV (%)	13.19	11.19	26.03	29.34	18.17
	DS3	80AW	27.2**a**	2.24**a**	9.53**a**	6.15**a**	21.60
		50AW	18.6**b**	1.78**b**	4.38**b**	2.75**b**	20.58
		20AW	16.1**c**	1.84**b**	3.35**c**	2.34**b**	24.55
			**	*	**	**	ns
		A. hybridus	21.4	2.29	5.84	3.34	20.12
		A. tricolor	19.8	1.61	5.67	4.16	24.36
			*	**	ns	ns	*
		CV (%)	10.56	21.97	25.27	37.34	26.20

In each section, means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; CV, coefficient of variation; 80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW; RS1, January -March 2014; RS2, September-November 2014; RS3, February-April 2015; DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015.

Similar results were found in other crops where the panicle and spike length were significantly reduced in rice (Akram et al. 2013) and wheat (Akram 2011) when the plants were submitted to water stress at the flower initiation and stem elongation stages respectively.

Results of panicle length and panicle internode length showed differences between species during all seasons. Longer panicle length and panicle internodes were developed by *A. hybridus* compared to *A. tricolor* as shown in Table 4.2. This difference may be attributed to the difference in their genetic variation.

Grain and straw yield

The WR significantly affected straw yield during RS3 and grain yield during RS2 and RS3 only. Both paramenters, however, were significantly affected by WR during all dry seasons. As shown in Table 4.2, although the panicle and panicle internode lengths have been affected by WR in all seasons, the straw and grain yields were not affected during RS1. It is clear that the water deficit was not high enough to affect straw and grain yields in either species due to sufficient SW at 50AW and 20AW treatments (Figure 4.2). However, due to a reduction in SW during RS2 and RS3, the grain yield decreased at 50AW and 20AW while the straw yield had a similar reduction only during RS3. During the dry season, the reduction in straw yield was similar to those observed during RS3 and the grain yield tended to decrease with decrease of the SW from 80% to 20% of AW (Table 4.2). Since the duration and severity of the water stress lead to a reduction in plant development, net photosynthesis and photoassimilate allocation to grain filling (Farooq et al. 2009, Anjum et al. 2011), the reduction in grain yield during all seasons was due to low SW occurring at different growth and development stages. A significant reduction was found in *A. cruentus* grain yield under permanent water stress conditions (Mlakar et al. 2012). However, Olufolaji et al. (2010) reported an improved grain yield in *A. cruentus* under moderate water stress compared to severe and unstressed conditions. This result differs from those found in the present study. The different effects of drought on grain yield across experiments were probably due to the different experimental conditions and species used.

Although WR significantly affected harvest index (HI) during RS2 and DS2, it did not affect the trait during other seasons (Table 4.2). In a pot experiment under greenhouse conditions, the HI of *A. cruentus* was not affected by water stress (Mlakar et al. 2012). Results from other crops also showed non-significant water stress effects on HI for example in wheat (Akram 2011). Increased HI has contributed to increasing grain yields of rice (Fageria et al. 2011) and wheat (White and Wilson 2006). The fact that there is insufficient evidence to support the WR effect on HI and the similar HI observed at 80AW and 20AW treatments in this study might be beneficial for grain production in both species during rainy and dry seasons.

The species differed significantly in straw and grain yields only during rainy seasons and in HI during RS1, RS3 and all dry seasons (Table 4.2). Although *A. hybridus* developed a high straw yield during RS1 and RS2, the highest grain yield was produced by *A. tricolor* during RS1 and RS3. The high straw yields in *A. hybridus* seem to be the result of the high SW recorded during RS1 and RS2 (Figure 4.2). The high grain yield of *A. tricolor* during RS1 and RS3 was developed under relative long periods of vegetative growth, 10 and 8 WAE, respectively (Figure 4.3B). This indicates that *A. tricolor* needs at least 8 weeks of vegetative growth to develop enough photosynthates for grain filling under the current study environment.

Table 4.3: Combined analysis of variance over sowing dates on grain yield and harvest index during rainy and hot season (**A**) and dry and cool season (**B**)

A. RAINY SEASON

Source	df	Grain yield ($g\ plant^{-1}$) Probability	Grain yield Mean	Harvest index (%) Probability	Harvest index Mean
Sowing date (SD)	2	0.0000**		0.0000**	
Repetitions within SD	15	0.0000**		0.0000**	
Watering regime (WR)	2	0.0005**		0.1779^{ns}	
80AW			12.15**a**		
50AW			11.13**b**		
20AW			9.35**c**		
SD x WR	4	ns		0.0100**	#
Species (S)	1	0.0578^{ns}		0.0000**	
A. hybridus					19.19
A. tricolor					21.59
SD x S	2	0.0085**	#	0.0761^{ns}	
WR x S	2	ns		ns	
SD x WR x S	4	ns		0.3119^{ns}	
Error	75				
Total	107				
Coefficient of variation		27.04%		13.05%	

B. DRY SEASON

Source	df	Grain yield ($g\ plant^{-1}$) Probability	Grain yield Mean	Harvest index (%) Probability	Harvest index Mean
Sowing date (SD)	2	0.0000**		0.0000**	
Repetitions within PD	15	ns		0.0439*	
Watering regime (WR)	2	0.0000**		0.2040^{ns}	
SD x WR	4	0.0000**		0.2304^{ns}	
Species (S)	1	ns		0.1245^{ns}	
SD x S	2	0.0590^{ns}		0.0011**	#
WR x S	2	0.3639^{ns}		ns	
SD x WR x S	4	0.0129*	#	ns	
Error	75				
Total	107				
Coefficient of variation		37.21%		24.53%	

Mean comparisons are presented in figure 4.4.
In each section, means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01 respectively; F, F value; 80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW.

In addition, the biomass allocation into the grain was higher in *A. tricolor* than in *A. hybridus* during RS1 and RS3 (Table 4.2). These findings suggest that *A. tricolor* is better adapted for grain production compared to *A. hybridus* during the rainy season in southern Mozambique.

The results of the combined analysis over sowing date (SD) show that the grain yield was significantly affected by the WR during the rainy season, indicating that the grain yield decreased at 50AW and 20AW (Table 4.3A). Beside that, a significant interaction with regard to grain yield was shown between SD and S, suggesting that the species responses differed at different SD (Figure 4.4A). As shown in Figure 4.4A, the grain yield was different among the species at all SD. *Amaranthus tricolor* produced higher grain yield in January sowing RS3 and December sowing RS1, and lower in August sowing RS2. Furthermore, the HI was significantly higher in *A. tricolor* than *A. hybridus* (Table 4.3A). These findings suggest that *A. tricolor* is better adapted for grain production during rainy seasons since the growth occurs at day lengths above 12 hours day^{-1} (Figure 4.3A). This seems to correspond with the facts stated by Christiansen et al. (2010) that short day plants are common in tropical areas and beneficial to crop development due to rainy seasons that coincide with the warmest period of the year.

The HI was affected by an interaction between SD and WR (Table 4.3A). Although the HI trend was not clear at different WR and SD, it was low at 20AW in the August sowing RS2 (Figure 4.4B) where the period of vegetative growth was lower compared to the December sowing RS1 and the January sowing RS3 (Figure 4.3B). Despite the low HI at 20AW, the allocation of photosynthates to filling the grain was highest in the August sowing RS2 for both species. The increase in HI followed the decrease in period of vegetative growth during the rainy season (Figure 4.3B). It seems that the HI is dependent upon time to flowering.

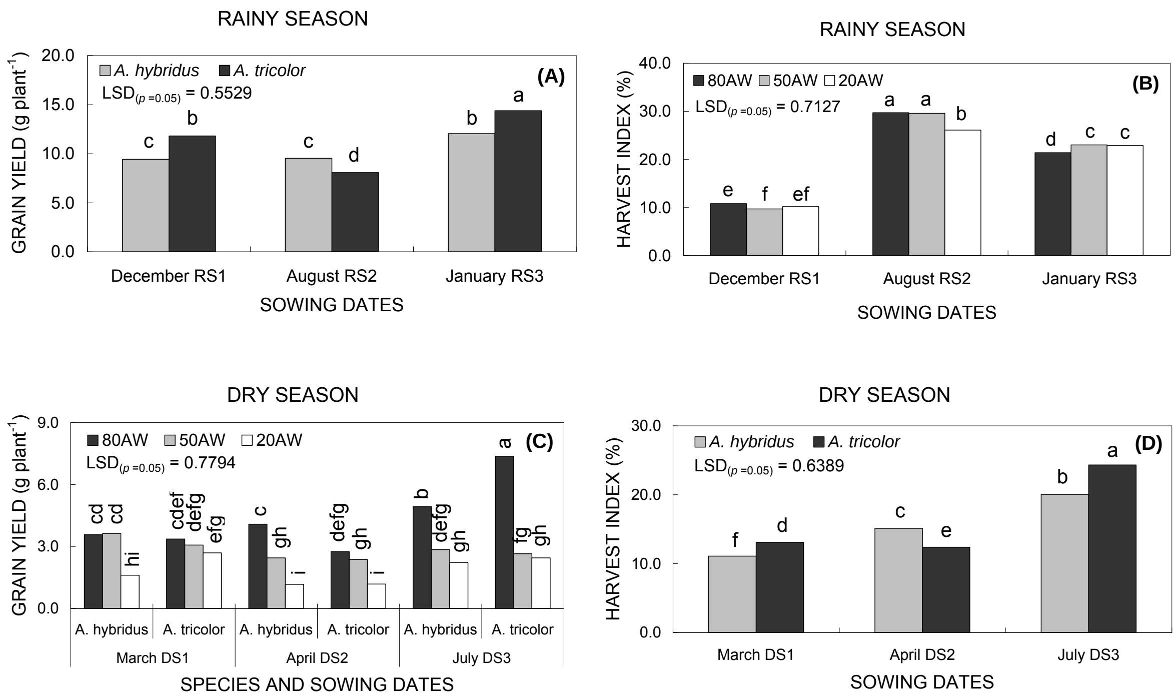

Figure 4.4: Interaction between (**A**) sowing date and species in terms of grain yield, (**B**) sowing date and watering regime (80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW) in terms of harvest index, (**C**) sowing date, watering regime and species in terms of grain yield, and (**D**) sowing date and species in terms of harvest index, during rainy (RS1, January-March 2014; RS2, September-November 2014; RS3, February-April 2015), and dry (DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015) seasons. Means followed by the same letters in each figure are not significantly different at 5% level probability (DMRT).

During the dry season, the grain yield was significantly affected by an interaction between SD, WR and S (Table 4.3B). Except for *A. tricolor* in the March sowing DS1, the grain yield tended to decrease significantly (p <0.05) with a decrease in SW from 80% to 20% of AW for both species at all SD (Figure 4.4C). Although above-ground biomass has been lower during DS3 compared to DS1 seasons as reported in

Chapter 3, the highest grain yield was obtained in the July sowing DS3 at 80AW for both species, with a significantly higher value showed in *A. tricolor*. However, the grain yield of *A. hybridus* was higher than *A. tricolor* in the April sowing DS2 but there was no significant difference between species in the March sowing DS1. On one hand the high grain yield developed by both species in the July sowing DS3 occurred at early flowering with a mean day length below 11 hours day^{-1} (Figure 4.3A). On the other hand, an interaction between SD and S significantly affected HI during dry season (Table 4.3B). As shown in Figure 4.4D, the HI was different among the species at all SD with the greatest values observed in *A. tricolor* except in the April sowing DS2. Furthermore, the HI was high for both species in July sowing DS3 where most of the biomass was synthesized only in 6 weeks of vegetative growth (Figure 4.3B). This suggests that the high grain yield of *A. hybridus* and *A. tricolor* observed in the July sowing DS3 was probably due to the high allocation of photosynthates into the grain since the limited photoassimilate is preferentially directed towards developing grain which is the sink with the greater strength (Hopkins 1999). It was also observed during the rainy season that the increase in HI (Table 4.3A) followed the decrease in above-ground biomass (Chapter 3).

The results from this study provide useful information on appropriate sowing dates and species which could be beneficial for amaranth grain production in southern Mozambique. In this region, the monthly mean day length increases from 11.95 to 13.66 hours day^{-1} in September to December, and then decrease to 12.22 hours day^{-1} in March. The grain yield ranged from 8.07 to 14.38 g plant^{-1} in both species during the rainy season, with minimum and maximum values observed in *A. tricolor* (Figure 4.4A). However during the dry season, the grain yield decreased to a range of the 2.75-7.37 g plant^{-1} observed at 80AW, with minimum and maximum values also showed in *A. tricolor* (Figure 4.4C). For grain production under rain-fed conditions with supplementary irrigation, it seems recommendable to sow *A. tricolor* from October to February during the rainy season, where it is expected that the plants are exposed to photoperiods above 12 hours day^{-1} for a relatively long time. Although *A. tricolor* had better performance in grain production compared to *A. hybridus* in the July sowing, there is not sufficient evidence to prove differences in performance between the species at photoperiods below 12 hours day^{-1}. Therefore, the sowing in other months of the year can be done with both species. Further study is needed to evaluate the response of these two species at photoperiods below 11 hours day^{-1} (May and June sowing) in terms of grain yield.

Grain crude protein

Neither WR nor species affected the grain crude protein (CP) content. However, the CP content showed differences between seasons varying from 7.16 to 18.57% in both species (Figure 4.5). Since nitrogen fertilization positively affects the protein content of amaranth grain (Olaniyi et al. 2008, Mlakar et al. 2010) and the fact that only 150 kg ha^{-1} of urea were applied during RS1 and DS1 instead of 225 kg ha^{-1} applied during RS2 and DS2, the low CP values showed in Figure 4.5 are probably the result of the low nitrogen amount available to the plants. The CP values obtained during RS2 and DS2 are within the scope of 13.1-21.0% as presented by Mlakar et al. (2010) and the 17.6-19.0% found in *A. hybridus* grain as reported by Dhellot et al. (2006). This finding suggests that both species offer a potential source of protein for local people and thus it might contribute to dietary diversification and food security.

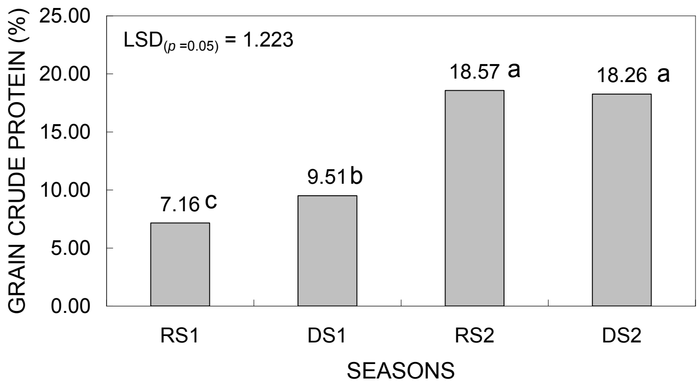

Figure 4.5: Grain crude protein content of *A. hybridus* and *A. tricolor* in different seasons (RS1, January -March 2014; DS1, April-June 2014; RS2, September-November 2014; DS2, May-July 2015). Means followed by the same letters are not significantly different at 5% level probability (DMRT).

Conclusions

The water limitations at 50% and 20% of available water led to a reduction in panicle and panicle internode length, and straw and grain yields in *A. hybridus* and *A. tricolor* during rainy and dry seasons. On the contrary, the time to flowering and grain crude protein of the two species were unaffected by the water deficit. It means that both species have the aptitude to be a source of proteins throughout the year.

Although the onset of flowering was determined by day length and minimum daily temperature, the day length was the most determinant factor. The increase in minimum temperature delayed flowering mainly when the day length was above 12 hours day^{-1} which occurred in the rainy season. The highest grain yield and harvest index was produced by *A. tricolor*. Both species have reacted like short day plants.

This study revealed that *A. tricolor* have the potential to be cultivated from October to February under rain-fed condition with a supplementary irrigation. During other months of the year, mainly in the dry season, both species showed potential to be cultivated under an irrigation schedule based on reference to a soil water content of 80% of available water. To conclude, it is achievable to cultivate *A. hybridus* and *A. tricolor* all year round if irrigation is available to ensure a soil water content at 80% of available water, and thus to contribute to food security and dietary diversification in southern Mozambique.

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Chapter 5

Amaranthus hybridus and *A. tricolor* leaf yield and nutrient contents as affected by different watering regimes and harvesting intensity in different seasons in southern Mozambique

Abstract

The use of amaranth as a leafy vegetable with high nutritional value can be part of the strategy to improve not only nutrition security but also food security in southern Mozambique. A study with six field trials (three during the rainy season and three in the dry season) in a randomized complete block design with 3 x 2 x 2 factorial layout with three replications were conducted in Maputo, Mozambique. The effect of harvesting intensity (25% and 50% of height removed) on vegetative growth, leaf yield and leaf nutritional content of *Amaranthus hybridus* and *A. tricolor* under different watering regimes (80%, 50% and 20% of total available water) were assessed. The leaf and side-shoot numbers, leaf area, aboveground yield as well as leaf calcium and crude protein contents were measured. This study showed insufficient evidence to conclude that the interaction effect of watering regimes and harvesting intensities have affected the vegetative growth of either species. However, the leaf and side-shoot numbers, leaf area and aboveground yield were sensitive to the decrease of soil water from 50% to 20% of available water in plants submitted to successive cuttings. The calcium and crude protein content in leaves were unaffected by either watering regime or harvesting intensity. *Amaranthus hybridus* showed better performance and higher leaf yield when the plants were topped by 25% of their height over a year.

Keywords: amaranth, harvesting intensity, leaf yield, watering regime

Introduction

The production of amaranth as a leafy vegetable well adapted to arid and semi-arid regions (Schahbazian et al. 2006) with high nutritional value (Allemann et al. 1996, Akubugwo et al. 2007, Andini et al. 2013) can be part of the strategy to improve food security and nutrition in southern Mozambique. Amaranth leaf yield is greatly affected by water and fertilizer application (Ejieji and Adeniran 2010) as well as by harvesting procedure (Grubben 1976, Mnzava and Masam 1985, Norman and Shongwe 1993, Diwani and Janssens 2001, Ribeiro and Combrink 2006). The plant may be harvested once by uprooting or cutting at the ground level, or several times by repeated leaf cutting (Grubben 2004, Ebert et al. 2011). In the latter case, the leaf yield reaction depends on the growing of leaves and buds that are left behind for re-growth (Mnzava and Masam 1985). As a result of multiple leaf cuttings, plants developed more aboveground biomass than uncut plants (Bello et al. 2011).

As vegetables, *A. hybridus* and *A. tricolor* appeared to be equally adapted to the different climatic conditions that occur during the rainy and dry seasons (Chapter 3). The rainy and hot season and dry and cool season are the two distinct seasons in southern Mozambique. In this region, the amaranth species are not widely cultivated but they are protected and collected regularly in the nature and in cultivated fields. Generally, the leaves are collected manually leaf by leaf or by cutting the top portion of main stems and thereafter side-shoots to consume as tender greens. Harvesting by uprooting is not common.

Several studies have described the vegetative growth of *A. hybridus* and *A. tricolor* in response to water stress and harvesting intensity (cutting height), however information on their interaction effect is scarce. A study on the leaf yield response to water deficit have indicated that these two species are sensitive to water deficit during the dry season but tolerate short and intermittent stress during the rainy season in southern Mozambique (Chapter 3). This was attributed to the rainfall pattern and mechanisms developed by *A. hybridus* and *A. tricolor* to reduce leaf water loss under drought stress (Slabbert et al. 2004). Apart from the tolerance to water deficit, Norman and Shongwe (1993) reported higher leaf yield with 15 and 20 cm initial cutting height compared to 10 cm in *A. hybridus*. However, Grubben (2004) stated that repeated cutting is less suitable for *A. tricolor* compared to other amaranth species. Since the length of time in which the plants are exposed to water stress and its severity affects the plant growth (Akinci and Lösel 2012) and the repeated cutting reduce leaf water loss under drought conditions, a suitable amount of leaves left after cutting may help the plant to recover and restore leaf hydration. Diwani and Janssens (2001) reported a reduction of the drought effect on the leaf dry mass accumulation after multiple leaf harvests in five cultivars of traditional leafy vegetables including *A. cruentus*. In a different study, Ribeiro and Combrink (2006) found that salt-stressed *A. tricolor* restored leaf yield to levels equal and superior to no-stressed plant with less destructive cutting of a 25% topping. Although those results cannot be extrapolated to the case of water-stressed plant, it might indicate that the use of harvesting procedure by removing leaves may also minimize the effect of limited water on leaf dry mass, since one of the effects of saline conditions is to reduce the uptake of water by the plant.

There is no information regarding the vegetative growth of *A. hybridus* and *A. tricolor* in response to the combined effect of water stress and harvesting intensity in Mozambique. This information could help the existing few and possibly future amaranth growers to plan a suitable harvesting intensity to arid and semi-arid regions of the south of the country. For this purpose, a study was conducted under field conditions to assess the effect of harvesting intensities and watering regimes on the vegetative growth, leaf yield and leaf nutritional content of *A. hybridus* and *A. tricolor* during the rainy and dry seasons in southern Mozambique.

Material and methods

Crop environment

A field experiment was carried out at the Faculty of Agronomy and Forestry Engineering (FAEF), Eduardo Mondlane University, experimental station in Maputo (25°57′ S, 32°34′ E; 60 m altitude) during six seasons; three in the rainy seasons: 15 January to 23 March 2014 (RS1), 23 September to 26 November 2014 (RS2), and 18 February to 23 April 2015 (RS3), and the other three in the dry seasons: 14 April to 22 June 2014 (DS1), 14 May to 19 July 2015 (DS2), and 11 August to 11 October 2015 (DS3). *Amaranthus hybridus* seeds used for these trials were collected in March 2013 from a producer field in Palma district (Northern Mozambique) and later multiplied in the fields of the FAEF, and *A. tricolor* seeds were obtained through the ARC-Roodeplaat (South Africa). The seeds were germinated in seedling trays filled with Hygrotech Seedling Mix. The seedlings grew inside a net-covered house for 29, 36, 29, 27, 31 and 39 days before transplanting for RS1, RS2, RS3, DS1, DS2 and DS3 respectively. Urea (46% nitrogen [N]) was applied at 14 and 21 days after sowing at a rate of 14.4 kg N ha^{-1}. At the transplanting day, twenty four seedlings were transplanted to a field plot spaced at 25 cm in row and 35 cm between rows. Each plot covered a small area of 2.1 m^2 (1.5 m x 1.4 m).

The soil texture of the experimental plots was sandy. Table 5.1 shows some physical and chemical characteristics of the soil. Three days before transplanting, a basal application was done with 35 kg ha^{-1} for each nutrient: nitrogen, phosphorus and potassium. The application was a mixture comprising urea (46% N), NPK$_{(12:24:12)}$ and potassium sulphate at a rate of 38, 146, and 35 kg ha^{-1} respectively. Nitrogen at a rate of 35 kg ha^{-1} was applied as top dressing in three equal splits at 15, 30 and 45 days after transplanting (DAT) using urea (46% N), except in RS1 and DS1 seasons where only two split applications (20 and 40 DAT) were done at the same rate of 35 kg N ha^{-1}.

Table 5.1: Some soil physical and chemical characteristics of the experimental station of Eduardo Mondlane University where the amaranth trial was carried out

Depth (cm)	Texture sand (%)	PWP	FC (mm)	TAW	$EC_{(1:2.5)}$ (mS cm^{-1})	pH_{H2O}	Organic matter (%)
0-20	90.1	10.1	22.4	12.3	0.09	6.85	0.50
20-40	91.7	10.1	24.2	14.1	0.08	6.99	0.07

PWP, permanent wilting point; FC, field capacity, TAW, total available water; EC, electrical conductivity

Mean temperature per day during the period of trials were recorded at the Mavalane International Airport weather station located within a radius of 5 km from the site trials (Table 5.2). The crops were harvested at 67, 64, 64, 69, 66 and 60 DAT for RS1, RS2, RS3, DS1, DS2 and DS3 respectively.

Table 5.2: Average daily temperature, total amount of rainfall and number of days of rain during the field trial periods

Seasons	RS1	RS2	RS3	DS1	DS2	DS3
Temperature (°C)	27.4	22.8	25.5	22.8	21.0	22.9
Rainfall (mm)	374.0	103.5	78.0	1.5	7.0	18.5
Number of days with rainfall	18	17	14	1	2	5

RS1, January-March 2014; RS2, September-November 2014; RS3, February-April 2015; DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015.

Treatments and experimental design

Treatments comprise two species (*A. hybridus* and *A. tricolor*), three levels of watering regimes (irrigated at 80% [80AW], 50% [50AW] and 20% [20AW] of total available water), and two levels of leaf harvesting intensity (low and high intensities) used in a 3 x 2 x 2 factorial experiment. The experiments were conducted in a randomized complete block design in a factorial arrangment in three replications. Each of the 2.1 m^2 plots was separated from one another by 1.5 m in order to prevent contamination between plots considering the water mobility in sandy soils in particular.

From 12 DAT to final harvest, the irrigation treatments were monitored based on soil water (SW) measured by a Time Domain Reflectometer (TDR) HydroSense II Water Content Sensor with 20 cm Rods (Campbell Scientific, Inc.). The TDR was previously calibrated in the trial site as reported in Chapter 3. The SW monitoring was conducted at 0-40 cm depth in order to cover the root zone where the most effective roots are located. It is worth to mention that no information regarding effective root depth of *A. hybridus* and *A. tricolor* plants has been found in the consulted literature. However, roots of *A. cruentus* reached depths of 13 and 35 cm in 1 and 3 months after planting respectively (Fasinmirin et al. 2008). Moreover, the taproot and many secondary roots tended to develop horizontally in the field due to the initial inhibition of geotropic development of the primary taproot forced by the seedling tray and the low capacity to recover its geotropic growth after transplanting (Egel and Martyn 2008). At 20-40 cm depth, the SW was measured as reported in Chapter 3.

Irrigation schedule based on SW content and measured every two days using a handheld TDR had the following procedure: from 12 to 30 DAT, the plants were irrigated when the SW of the first layer (0-20 cm depth) reached 20.0, 16.4 and 12.7 mm for 80AW, 50AW and 20AW treatments respectively; after 30 DAT, 41.4, 33.5 and 25.6 mm of SW in the top 40 cm were used as indicators to irrigate. In order to guarantee these SW pre-defined levels of 80AW, 50AW and 20AW treatments, the amount of 6.2 mm of water were applied at irrigation intervals varying from one to two days, three to four and five to six days respectively. The experimental plots were watered by hand using a watering can of 13 L capacity. Three rain gauges placed in field trials were used to record the rainfall (Table 5.2).

The treatments of leaf harvesting intensity started at 25 DAT by cutting the top part of the main stem. The low intensity treatment, where 25% of the height was removed, and the high intensity treatment, where 50% of the height was removed, were applied during first harvest; afterwards all side-shoots longer than 3 cm from the main stem node were topped at the same intensities. The leaf harvesting was done every two weeks for eight weeks. In the fourth and last harvest the entire aboveground plant was removed.

Data collection

Data collection was done on the four central plants of each plot for all seasons to prevent border effects. All measurements were done on aboveground plants. The plant heights (H_0 and H_1), from the soil level to the youngest fully expanded apex leaf, were measured 6 and 21 DAT using a tape measure. The initial height growth used as covariate was calculated by the difference between H_1 and H_0. The collected leaves were counted in each harvest and the sum of the all harvests was used as final leaf number. From the second harvest onwards, the same procedure was used to calculate the side-shoot number. The length and width of 7 randomly selected leaves of the 4 central plants from each harvest were measured with a tape measure. The leaf area was calculated using a linear equation (Kvet and Marshall, 1971):

Leaf area (cm^2) = 0.654 x (L x W);

Where: 0.654 = leaf shape coefficient; L = length of leaf (cm); W = width of leaf (cm) measured at half length.

In each harvest, the mean leaf area was used as leaf size and the total leaf area was calculated by multiplying leaf size with leaf number. The final leaf area was the average of the four total leaf areas of the harvests. The leaves and stems (main stem, side-shoots and trace of inflorescence in some cases) cut during the first three harvests were dried at 80°C for 48 hours and their dry mass were determined. In the last harvest, the removed aboveground plants were divided into leaves and stems, and also dried to determine their dry mass. The final leaf and stem dry mass were calculated by the sum of all harvesting and the aboveground biomass by adding the final leaf dry mass and stem dry mass. The leaf:stem (L/S) ratio was calculated by dividing the leaf dry mass by stem dry mass. Leaf calcium and nitrogen contents were analyzed at the Soil Laboratory of the FAEF. The samples consisted of a mixture of dry leaf samples of the three trial replications per treatment and the laboratorial analysis was replicated during RS1, RS2, DS1 and DS2 seasons. In each season, samples were collected at first and final harvest. The Complexometric

Titration Method after digestion with nitric and perchloric acids (Waling et al. 1989) and Kjeldahl Method were used to determine calcium content and total nitrogen in leaves respectively. By multiplying nitrogen content with the factor 6.25 the leaf crude protein (CP) content was calculated.

Data analysis

Covariance analysis was used in order to control experimental error and to adjust treatment means. Initial height growth was used as a covariate. The data, except calcium and crude protein content, were subjected to analysis of covariance using the MSTAT-C Version 1.2 computer program. Duncan´s New Multiple Range Test (DMRT) was used to compare treatment adjusted means. The analysis of covariance was individually performed for each trial. To compare diverse climatic conditions and measure the response to treatments at different transplanting date (TD) a combined analysis of covariance over TD was performed for each season (rainy and dry).

The calcium and crude protein content were subjected to analysis of variance (ANOVA) using the same statistic computer program and DMRT was also used to compare treatment means. To compare different stage of plant development and measure the response to treatments at different harvesting times a combined analysis of variance over harvesting time was performed with species, watering regime and harvesting time as factors and season as replication.

Results and discussion

As shown in Figure 5.1, the soil water (SW) exceeded the intended watering regimes (WR) during the rainy season due to the higher amount of recorded rainfall. It was particularly evident during RS1. However, during the dry season, the SW values were around the intended WR guidelines.

An interaction between WR and species (S) was only significant for leaf number and leaf yield during DS2. The leaf number and total leaf area were only affected by an interaction between WR and harvesting intensity during DS2; a similar interaction was also found for L/S ratio during RS2 and DS2. Due to those inconsistent results, the discussion will be focused on the main effects of WR, S and harvesting intensity.

Vegetative growth

Leaf size was the only trait significantly affected by WR during RS3 in the rainy season (Table 5.3). However, the WR affected leaf number and leaf area during all dry seasons while the side-shoot number was only affected during DS3. The leaf size was reduced with a decrease in SW from 50% to 20% of AW, except during DS3 where the reduction was observed from 80% to 50% of AW (Table 5.3). The reduction in leaf number, total leaf area and side-shoots number during dry seasons followed the reduction in leaf size. This result is in agreement with Anjum et al. (2011) and Akinci and Lösel (2012) who stated that plants will react to limited water availability with a reduction in leaf canopy to reduce water loss. The fact that the WR affected leaf size but not the leaf number and total leaf area during RS3 season, indicates that the leaf size is more sensitive to water deficit since SW values were around the intended WR compared to RS1 and RS2 (Figure 5.1).

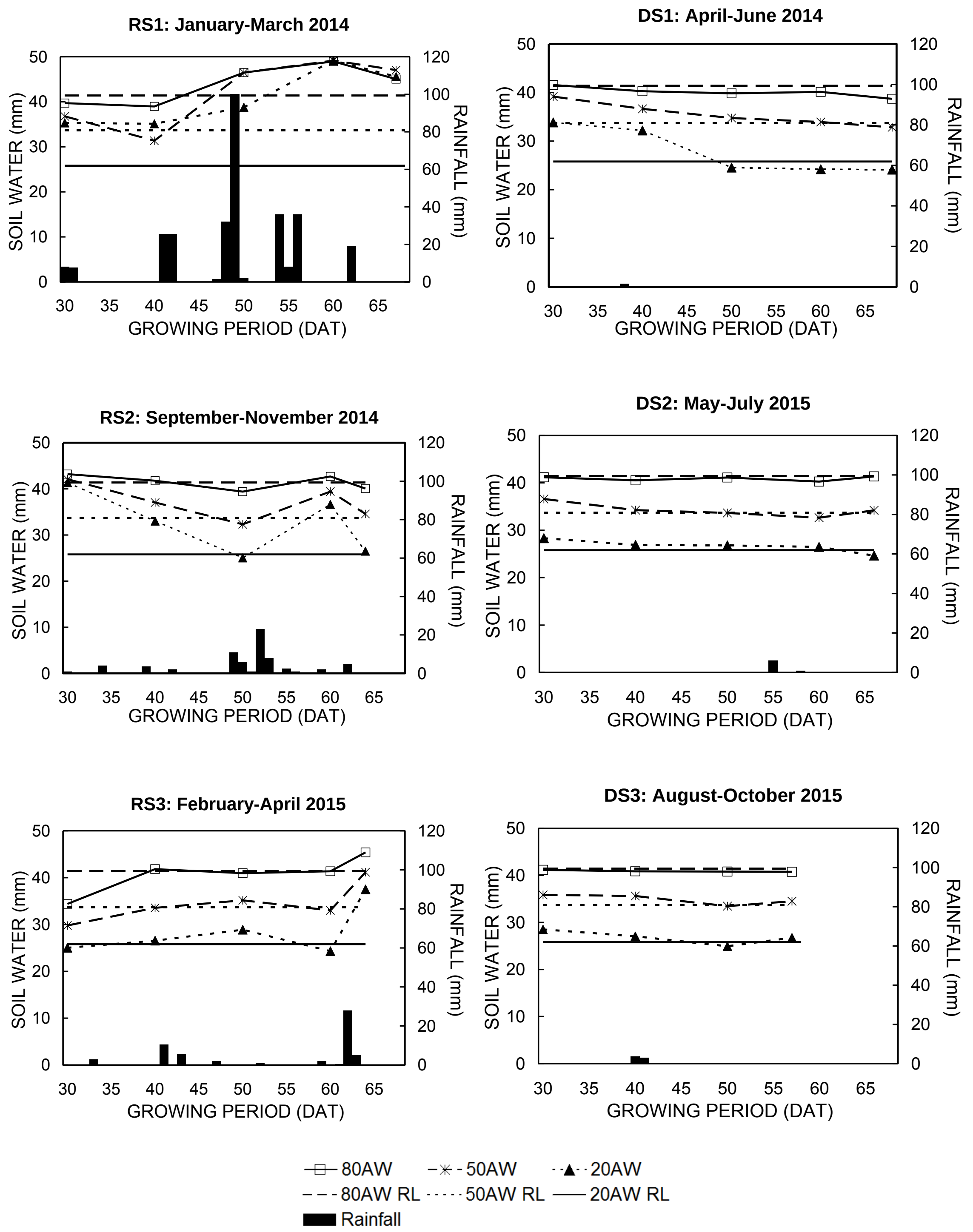

Figure 5.1: Rainfall and soil water (average of every 10 days) measured in the top 40 cm of soil during the different trial periods. The three required levels (RL) of watering regimes (WR) are shown (80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW).

This is also in line with Blum (2011) who argued that under drought stress plants can maintain the same leaf number but the leaves are smaller because the organ differentiation seems to be more resilient to water deficit than expansion growth.

Amaranthus tricolor developed smaller leaves but both species showed similar leaf and side-shoot numbers as well as total leaf area during rainy seasons as shown in Table 5.3. During dry seasons, apart from the smaller leaves, the total leaf area was lower in *A. tricolor* compared to *A. hybridus*, except during DS3. The high total leaf area showed in *A. hybridus* was due to larger leaf size developed during DS1 and DS2 since leaf and side-shoot numbers were similar between species. It may indicate that *A. hybridus* is better adapted as leafy vegetables with repeated leaf harvesting during dry seasons.

The main effect of harvesting intensity significantly affected side-shoot number and leaf size during rainy seasons and leaf and side-shoot numbers, as well as leaf area during dry seasons. Except in DS3, plants reduced by 25% (plants whose 25% of their heights measured from apex were topped at every harvest) showed more side-shoots and larger leaves during rainy and dry seasons, and higher leaf number and total leaf area during dry seasons (Table 5.3). Norman and Shongwe (1993) also found an increase in side-shoots number with initial cutting height of 20 cm compared to 15 and 10 cm in *A. hybridus*. Different result was found in *A. tricolor* under soilless systems, plants topped by 25% and 50% did not differ in number of newly formed side-shoots (Ribeiro and Combrink 2006).

Table 5.3: Main effects of the watering regime, amaranth species and harvesting intensity on leaf and side-shoot number, leaf size and leaf area of amaranth leafy vegetables grown at the experimental station of Eduardo Mondlane University

Parameters	Treatments	Rainy seasons			Dry seasons		
		RS1	RS2	RS3	DS1	DS2	DS3
Leaves (number)	80AW	246.7	353.3	394.2	167.4**a**	202.8**a**	299.4**a**
	50AW	261.8	401.8	378.3	175.2**a**	220.1**a**	246.3**ab**
	20AW	259.1	330.6	348.1	145.6**b**	171.8**b**	232.4**b**
		ns	ns	ns	*	**	*
	A. hybridus	261.2	353.5	371.6	167.9	194.1	241.1
	A. tricolor	250.5	370.3	375.4	157.6	202.4	277.6
		ns	ns	ns	ns	ns	ns
	25%	267.3	376.7	365.9	175.2	208.3	293.2
	50%	244.4	347.2	381.1	150.3	188.2	225.5
		ns	ns	ns	**	*	**
	CV (%)	25.01	20.03	16.61	12.89	11.67	19.59
Side-shoots (number)	80AW	32.9	45.5	43.1	20.4	27.1	40.9**a**
	50AW	37.7	53.1	41.6	22.7	29.1	32.5**b**
	20AW	34.1	42.8	39.3	21.0	25.4	28.5**b**
		ns	ns	ns	ns	ns	**
	A. hybridus	35.4	46.1	41.5	22.2	27.5	32.1
	A. tricolor	34.3	48.2	41.2	20.5	27.4	35.7
		ns	ns	ns	ns	ns	ns
	25%	40.4	52.0	44.3	24.5	29.3	39.8
	50%	29.4	42.2	38.4	18.3	25.1	28.1
		**	*	*	**	**	**
	CV (%)	24.42	22.59	18.59	16.99	15.30	16.17
Leaf size (cm^2)	80AW	25.4	18.0	22.0**a**	13.9**a**	12.0**a**	12.9**a**
	50AW	25.5	18.9	22.1**a**	14.6**a**	12.6**a**	10.1**b**
	20AW	23.5	16.1	19.5**b**	11.7**b**	9.8**b**	8.6**b**
		ns	ns	*	*	*	**
	A. hybridus	27.4	19.0	22.1	14.7	13.1	11.0
	A. tricolor	22.2	16.4	20.3	12.1	9.8	10.1
		**	*	*	**	**	ns
	25%	26.7	18.7	22.3	14.3	12.4	10.7
	50%	23.0	16.6	20.1	12.5	10.5	10.4
		*	*	**	*	*	ns
	CV (%)	16.19	16.00	10.94	18.27	21.35	16.76
Total leaf area (cm^2 $plant^{-1}$)	80AW	6408	6628	8986	2844**a**	2765**a**	3935**a**
	50AW	6969	7678	8649	2995**a**	2897**a**	2535**b**
	20AW	6461	5600	7022	1700**b**	1788**b**	2095**b**
		ns	ns	ns	**	**	**
	A. hybridus	7331	6970	8463	2833	2754	2808
	A. tricolor	5894	6301	7976	2193	2213	2902
		ns	ns	ns	**	*	ns
	25%	7506	7281	8214	2754	2867	3294
	50%	5719	5990	8225	2273	2100	2416
		ns	ns	ns	*	**	**
	CV (%)	40.38	29.06	24.32	25.17	26.34	29.01

In each section, adjusted means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; CV, coefficient of variation; 80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW; 25%, plant topped by 25% of stem height; 50%, plant topped by 50% of stem height; RS1, January-March 2014; RS2, September-November 2014; RS3, February-April 2015; DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015.

Since half of leaves and buds were removed from the plants topped by 50%, the low leaf and side-shoot numbers and leaf area was probably due to limited reserves in the remaining shoots (leaves and branches) for plant re-growth, as was also reported by Mnzava & Masam (1985). On the other hand, the leaf number and total leaf area were not affected by the harvesting intensity during the rainy season. It appears that the available carbohydrate was enough for similar re-growth at both harvesting intensities, since optimal light intensity, temperature and water availability occurs in the rainy season and these are the foremost factors influencing the rate of photosynthesis. These results suggest that both harvesting intensities can be used during rainy seasons while the light harvesting intensity (topped by 25%) is recommended during dry seasons.

Aboveground yield components

Results of aboveground biomass showed significant differences among WR; it tended to decrease from 50% to 20% of AW during RS3 and the dry seasons (Table 5.4). Similar reductions were found in leaf and stem yields but reduced stem yields was only observed during dry seasons. It is noteworthy here that during dry seasons, plants in similar soil and climatic conditions without successive leaf cuttings, showed a reduction of aboveground biomass and leaf yield from 80% to 20% of AW as reported in Chapter 3. This might be an indication of a reduced drought effect on leaf dry mass due to multiple harvests as reported by Diwani and Janssens (2001). During the dry season, the reduction in aboveground biomass, leaf and stem yields was consistent with reductions observed in leaf number and leaf area.

Table 5.4: Main effects of the watering regime, amaranths species and harvesting intensity on aboveground yield components and leaf:stem (L/S) ratio of amaranth leafy vegetables grown at the experimental station of Eduardo Mondlane University

Parameters On dry mass basis	Treatments	Rainy seasons RS1	RS2	RS3	Dry seasons DS1	DS2	DS3
Aboveground	80AW	38.2	28.2	27.6**a**	8.7**a**	9.7**a**	14.2**a**
Biomass	50AW	35.8	32.2	24.7**ab**	9.0**a**	9.9**a**	9.6**b**
(g plant^{-1})	20AW	32.2	24.9	20.4**b**	6.5**b**	6.7**b**	8.0**b**
		ns	ns	*	*	**	**
	A. hybridus	38.1	30.5	25.2	8.9	9.4	10.5
	A. tricolor	32.7	26.4	23.3	7.2	8.2	10.8
		ns	ns	ns	*	ns	ns
	25%	42.1	31.5	24.7	9.2	10.0	12.2
	50%	28.7	25.4	23.7	6.9	7.6	9.0
		**	*	ns	**	**	**
	CV (%)	28.95	29.17	20.22	22.30	24.71	27.86
Leaves	80AW	20.1	14.3	17.4**a**	5.5**a**	6.0**a**	8.5**a**
(g plant^{-1})	50AW	18.8	16.6	15.8**ab**	5.7**a**	6.3**a**	5.5**b**
	20AW	17.1	12.7	13.0**b**	4.4**b**	4.3**b**	4.7**b**
		ns	ns	*	*	**	**
	A. hybridus	20.3	15.6	16.1	5.8	6.0	6.2
	A. tricolor	17.0	13.5	14.7	4.6	5.1	6.3
		ns	ns	ns	**	*	ns
	25%	20.6	15.1	15.3	5.6	6.0	6.9
	50%	16.7	14.0	15.4	4.8	5.0	5.5
		*	ns	ns	*	*	*
	CV (%)	26.34	25.66	18.18	18.91	20.50	27.14
Stems	80AW	18.1	14.0	10.2	3.2**a**	3.7**a**	5.6**a**
(g plant^{-1})	50AW	17.0	15.6	8.9	3.3**a**	3.7**a**	4.1**b**
	20AW	15.2	12.2	7.4	2.2**b**	2.4**b**	3.4**b**
		ns	ns	ns	*	*	**
	A. hybridus	17.8	14.9	9.1	3.1	3.4	4.3
	A. tricolor	15.7	12.9	8.6	2.7	3.1	4.4
		ns	ns	ns	ns	ns	ns
	25%	21.5	16.5	9.4	3.6	4.0	5.3
	50%	12.0	11.4	8.3	2.2	2.5	3.5
		**	**	ns	**	**	**
	CV (%)	32.39	34.40	28.01	32.96	35.95	30.90
L/S ratio	80AW	1.16	1.18	1.92	1.98	1.88	1.60
	50AW	1.22	1.11	1.94	1.99	1.85	1.41
	20AW	1.21	1.14	1.99	2.11	1.90	1.49
		ns	ns	ns	ns	ns	ns
	A. hybridus	1.22	1.13	1.89	2.02	1.98	1.48
	A. tricolor	1.17	1.16	2.00	2.03	1.78	1.52
		ns	ns	ns	ns	ns	ns
	25%	0.96	0.96	1.80	1.64	1.74	1.35
	50%	1.42	1.33	2.09	2.42	2.01	1.65
		**	**	*	**	*	**
	CV (%)	8.66	10.84	16.06	17.71	17.49	14.76

In each section, adjusted means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; CV, coefficient of variation; 80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW; 25%, plant topped by 25% of stem height; 50%, plant topped by 50% of stem height; RS1, January-March 2014; RS2, September-November 2014; RS3, February-April 2015; DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015.

The L/S ratio was not affected by WR in both seasons (rainy and dry). Since L/S ratio is an important factor affecting leaf quality in term of nutrient contents, this result indicates that the leaf quality of these amaranth species is not changed under 20AW conditions with successive leaf cuttings over the year in southern Mozambique.

Aboveground biomass, leaf and stem yield, and L/S ratio were similar in both species during most of the trial seasons (Table 5.4). However, the leaf yield of *A. hybridus* was higher than *A. tricolor* during DS1 and DS2 as well as aboveground biomass during DS1. These results might be explained by the largest total leaf area found in *A. hybridus* during the same seasons. It seems as if *A. hybridus* with repeated leaf cutting was better adapted as a leafy vegetable during dry seasons.

Except during RS3, the aboveground biomass showed significant differences between harvesting intensity during rainy and dry seasons; it was high in plants topped by 25%. Similar results were found in leaf and stem yields but the reduced leaf yield was not observed during RS2. The L/S ratio was high in plants topped by 50% during all the seasons. It was clear that the higher intensity harvesting promoted the allocation of limited photosynthates to the leaves, rather than to side-shoots.

Results of combined analysis over transplanting date (TD) show that the effect of interactions did not affect leaf yield and also there is no significant difference between TD during rainy seasons (Table 5.5). However, the main effect of WR, S and harvesting intensity significantly affected leaf yield. The highest leaf yields (16.96 and 17.60 g plant^{-1}) were achieved when these two species were submitted to 80AW and 50AW conditions. It is noteworthy here that the amount of rainfall received was high and probably relieved the adverse effect of drought stress on leaf yield, mainly during RS1 and RS2 (Figure 5.1). *Amaranthus hybridus* produced more leaf yield (17.20 g plant^{-1}) than *A. tricolor* (15.18 g plant^{-1}). The best leaf yield (17.01

g plant^{-1}) was reached in plants submitted to low intensity harvesting of 25%. This is in line with results found in *A. hybridus* (Norman and Shongwe 1993) and *A. cruentus* (Mnzava and Masam 1985, Akanbi et al. 2009).

Table 5.5: Summary of combined analysis of covariance over transplanting date on leaf yield during the rainy and dry seasons

Source	df	RAINY SEASON		DRY SEASON	
		Probability	Leaf yield (g plant^{-1})	Probability	Leaf yield (g plant^{-1})
Transplanting date (TD)	2	0.0852^{ns}		0.0000**	
Repetitions within TD	6	0.0205*		0.0028**	
Watering regime (WR)	2	0.0010**		0.0000**	
80AW			17.60**a**		
50AW			16.96**a**		
20AW			14.01**b**		
TD x WR	4	0.1934^{ns}		0.0009**	#
Species (S)	1	0.0140*		0.0021**	
A. hybridus			17.20		
A. tricolor			15.18		
TD x S	2	ns		ns	
WR x S	2	ns		0.0261*	#
TD x WR x S	4	ns		ns	
Harvesting intensity (I)	1	0.0403*		0.0005**	
25%			17.01		6.15
50%			15.38		5.17
TD x I	2	0.1021^{ns}		ns	
WR x I	2	ns		ns	
TD x WR x I	4	0.3569^{ns}		0.2562^{ns}	
S x I	1	ns		ns	
TD x S x I	2	ns		ns	
WR x S x I	2	ns		ns	
TD x WR x S x I	4	0.3775^{ns}		0.4140^{ns}	
Covariate	1				
Error	65				
Total	107				
Coefficient of variation		24.38%		24.14%	

#, Mean comparisons are presented in Figure 5.2.
In each section, adjusted means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; * and **, represent significance at $P \le 0.05$ and $P \le 0.01$, respectively; 80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW; 25%, plant topped by 25% of stem height; 50%, plant topped by 50% of stem height.

During dry seasons a significant interaction with regard to leaf yield was shown between TD and WR (Table 5.5), suggesting that responses differed at different TD. Although the degree of response did differ as shown in Figure 5.2A, leaf yield decreased with a decrease in SW from 80% to 50% of AW in DS3 and from 50% to

20% of AW in DS2. In DS1, the WR did not affect leaf yield. The highest leaf yield obtained at 80AW condition ranged from 4.23 to 9.25 g plant^{-1} in DS1 and DS3 respectively (Figure 5.2A).

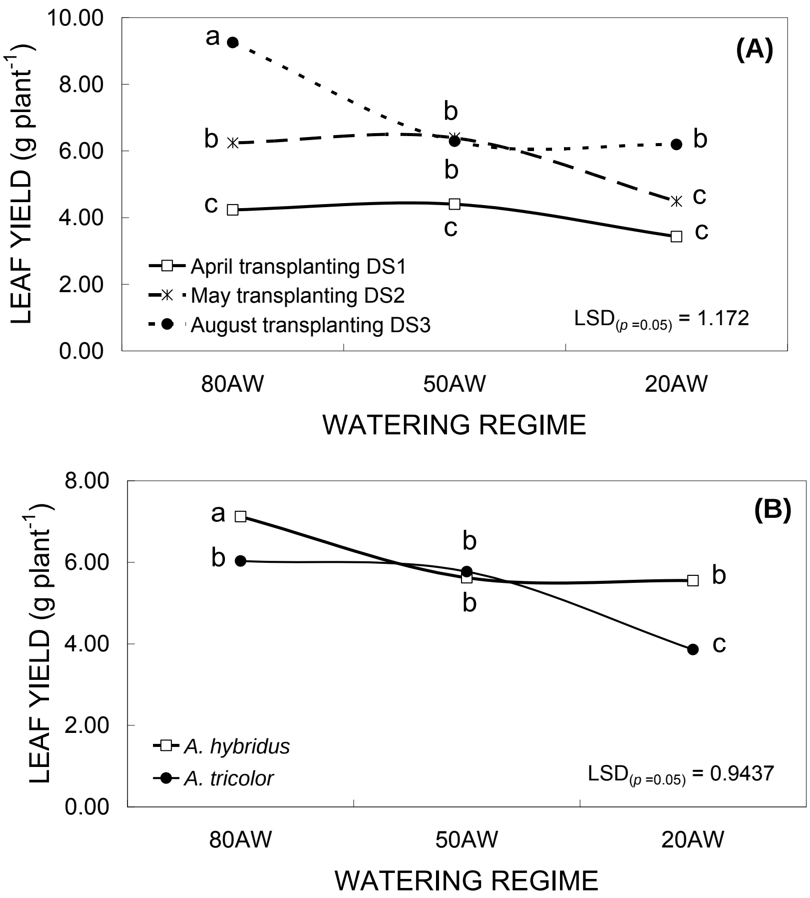

Figure 5.2: Interaction between (**A**) watering regime (80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW) and transplanting date and (**B**) watering regime and species affecting the leaf yield during dry season (DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015). Means followed by the same letters in each figure are not significantly different at 5% level probability (DMRT).

In Chapter 3 where the plants grew in similar growth season and soil characteristics but without successive leaf cuttings, the leaf yield was higher in DS1 than in DS3. That result differs from those found in the present study probably due to successive cuttings of leaves and side-shoots which prolonged the vegetative growth phase (Palada and Chang 2003, Awe and Osunlola 2013). This is very relevant for amaranth production as leafy vegetables, since short days induce early flowering during dry seasons in the south of Mozambique (Chapter 3).

Beside that, a significant interaction with regard to leaf yield was shown between WR and S in the dry season, suggesting that the species responses differed at different WR (Table 5.5). As shown in Figure 5.2B, the leaf yield was different between species at 80AW and 20AW conditions with the highest values (7.12 g plant^{-1} at 80AW and 5.55 g plant^{-1} at 20AW) observed in *A. hybridus*. The leaf yield decreased only with a decrease in SW from 80% to 50% of AW in *A. hybridus* and from 50% to 20% of AW in *A. tricolor*. This suggests that *A. hybridus* is better adapted as leafy vegetables under the highest and lowest SW levels with repeated leaf cuttings during dry seasons. Plants topped by 25% showed the highest leaf yield (6.15 g plant^{-1}) during dry seasons (Table 5.5).

Calcium and crude protein contents in the leaves

The calcium and crude protein content in leaves were affected by harvesting time. The leaf calcium was higher at final compared to first harvests (Figure 5.3A). Since the samples of final harvest included new and old leaves, the high calcium content observed is probably due to calcium accumulated in old leaves. Contrasting results were found in leaf crude protein; it decreased from 18.3% at first to 12.7% at final harvest (Figure 5.3B). These findings are in agreement with results reported by Modi

(2007) who found that the calcium content increased and crude protein content decreased in leaves of *A. hybridus* and *A. tricolor* with plant age. The high leaf calcium content (2724 mg 100 g^{-1}) found at final harvest is much lower than those reported in *A. tricolor* and *A. hybridus* without repeated cuttings (plants harvested once at maturity stage) while the low crude protein (12.7%) is within the range (12.67-14.74%) found in the same study (Chapter 3). Boukari et al. (2001) reported higher leaf calcium content of 3590 mg 100 g^{-1} in *Amaranthus* spp. compared to others western African leafy vegetables such as *Hibiscus esculentus* (2850 mg 100 g^{-1}), *Allium cepa* (2540 mg 100 g^{-1}), *Adansonia digitata* (2240 mg 100 g^{-1}) and *Solanum macrocarpon* (1590 mg 100 g^{-1}). According to Mnkeni et al. (2007), leaves of amaranth species contains three times more calcium compared to spinach.

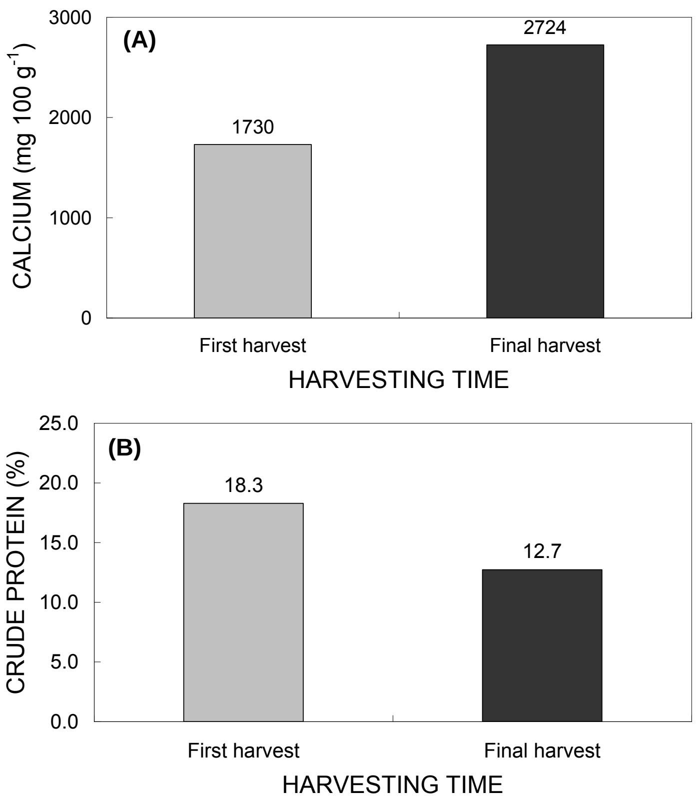

Figure 5.3: Leaf calcium (**A**) and crude protein (**B**) contents in leaves of *A. hybridus* and *A. tricolor* affected by harvesting time under different watering regime and harvesting intensity.

Since WR and harvesting intensity did not affect calcium and crude protein content in leaves of these two amaranth species, the amaranth leaves can be a supplement of calcium and protein in the main staple diet, and hence help efforts towards nutrition security in arid and semi-arid regions of southern Mozambique.

Conclusions

The results of the six-season experimental study showed insufficient evidence to conclude that the interaction effect of watering regimes and harvesting intensities affect vegetative growth of *A. hybridus* and *A. tricolor*. They also revealed that vegetative growth of both species are sensitive to the decrease of soil water from 50% to 20% of available water when submitted to multiple harvests (plants topped by 25% and 50% of their heights). However, the calcium and crude protein content in leaves were not affected by either watering regime or harvesting intensity but were affected by harvesting time.

This study provide relevant information for amaranth growers to select the amaranth species and plan a suitable harvesting intensity in arid and semi-arid regions. Under the conditions experienced in these trials, *A. hybridus* exhibited better performance and higher leaf yields was achieved when the plants were topped 25% of their height throughout the year. In addition, amaranth species appear to be a stable and good supplement of calcium and protein to improve nutrition security in southern Mozambique.

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Chapter 6

Influence of harvesting frequency on *Amaranthus hybridus* and *A. tricolor* leaf yield and nutrient contents under different watering regimes in different seasons in southern Mozambique

Abstract

Where most common exotic leafy vegetables are inaccessible, nutritive amaranth might be an alternative crop to improve nutrition security and nutrition in arid and semi-arid regions of southern Mozambique. A factorial experiment (2 x 3 x 2) was carried out to assess the influence of harvesting frequency on vegetative growth, leaf yield and leaf nutrient content of *Amaranthus hybridus* and *A. tricolor* under different watering regimes. The field trials laid out in a randomized complete block design with three replications were conducted in Maputo over six seasons. Growth and yield parameters such as leaf and side-shoot numbers, leaf area and leaf calcium and crude protein contents were examined. This study revealed that the vegetative growth of both species were tolerant to water deficits at 50% of total available water when successive harvests were made at 2 or 3 week intervals, and the highest leaf yield was obtained from *A. hybridus* which grows naturally in Mozambique. The leaf calcium content increased and the crude protein decreased with the age of the plant. The highest crude protein content was found in *A. tricolor*. *Amaranthus hybridus* proved to be well adapted to those climatic conditions with harvests at two weeks intervals and if cultivated as a crop will help efforts to achieve nutrition and food security in southern Mozambique.

Keywords: amaranth, harvesting frequency, leaf yield, watering regime

Introduction

Amaranth is a C_4 dicotyledonous plant widely distributed in the tropics (Ebert et al. 2011) with an ability to adapt to drought stress (Liu and Stützel 2004) and which can be cultivated successfully in arid and semi-arid regions (Schahbazian et al. 2006). As a leafy vegetable, amaranth species can tolerate temperatures up to 40ºC (Ebert et al. 2011) and can be an alternative crop in regions and seasons where exotic leafy vegetables such as kale, cabbage and lettuce are unavailable. The consumption of amaranth leaves with high quality protein (Andini et al. 2013) and appreciable amount of mineral elements and vitamins (Akubugwo et al. 2007) can alleviate nutritional deficiencies and hence improve food security and nutrition in Mozambique.

Amaranth species, when harvested several times, are more productive than plants harvested once (Bello et al. 2011), consequently they can provide a continuous source of nutritious leaves to enrich the staple food over time. Multiple harvests may also alleviate the drought effect on leaf yield as was reported for the case of *Amaranthus cruentus* (Diwani and Janssens 2001) which is particularly relevant to arid and semi-arid regions of Mozambique.

According to the Köppen classification, southern Mozambique is characterized by a tropical dry savanna (BS) for the biggest part of the inland (INGC et al. 2003) and two distinct seasons are recognized: a rainy and hot season and a dry and cool season. The mean annual temperature range from 23 to 26 ºC and the average annual rainfall varies from 500 to 600 mm inland and 350 mm in the driest interior

(Reddy 1986, FAO 2005). In the region, amaranth species grows naturally or in cultivated fields as a weed principally during the rainy season. The leaves are often collected by topping to be consumed as tender greens, permitting repeated harvesting. Although few growers produce amaranth in small areas or home gardens, the practice of leaf harvesting and its frequency have not been evaluated and tested yet. This frequency is however a crucial factor on the productivity of most amaranth crops. Two and three weeks harvesting intervals have often been studied and reported in literature for amaranth as a leafy vegetable (Grubben 1976, Norman and Shongwe 1993, Materechera and Medupe 2006, Ebert et al. 2011).

The occurrence of *A. hybridus* in Mozambique is reported by Silva et al. (2004) however there is no indication that *A. tricolor* occurs naturally in the country. *Amaranthus tricolor* is considered one of the superior leafy vegetable species (Daloz and Munger 1980) and is well known in Asia (Grubben 2004). It is noteworthy here that these two species proved to be well adapted to rainy and dry seasons in southern Mozambique (Chapter 3). With regard to leaf harvesting frequency, Materechera and Medupe (2006) recommended leaves to be harvested every two weeks in *A. hybridus* while Grubben (2004) mentioned only two harvests for *A. tricolor* with three weeks interval for re-growth. In the latter case, he also stated that the multiple harvests are less suitable for *A. tricolor* if compared to other amaranth species.

The leaf cutting frequency combined with the time period that plants can withstand water deficient conditions without a significant loss is one of the issues that have to be managed in order to increase the leaf yield. Insight into this issue can help to plan a suitable harvesting frequency and therefore increase the leaf productivity. For this purpose, this study aimed to assess the effect of harvesting frequencies and watering regimes on vegetative growth, leaf yield and leaf nutritional content of *A. hybridus* and *A. tricolor* during the rainy and dry seasons in southern Mozambique.

Material and methods

A factorial experiment (2 x 3 x 2) with two species (*A. hybridus* and *A. tricolor*), three levels of watering regimes (irrigated at 80% [80AW], 50% [50AW] and 20% [20AW] of total available water [TAW]) and two leaf harvesting frequencies (every two weeks and every three weeks) was conducted at the experimental station of the Faculty of Agronomy and Forestry Engineering (FAEF), Eduardo Mondlane University, in Maputo (25°57′ S, 32°34′ E; 60 m above sea level) over six seasons. Three experiments were conducted during the rainy seasons (RS): from 15 January to 23 March 2014 (RS1), 23 September to 26 November 2014 (RS2), and 18 February to 23 April 2015 (RS3), and the other three experiments during the dry seasons (DS): from 14 April to 22 June 2014 (DS1), 14 May to 19 July 2015 (DS2), and 11 August to 11 October 2015 (DS3).

Field trials were laid out in a randomized complete block design with three replications. Each experimental plot of 2.1 m^2 (1.5 m x 1.4 m) comprised twenty four plants spaced at 25 cm in row and 35 cm between rows. A lane of 1.5 m between plots was left bare to prevent water contamination from one plot to another.

From 12 days after transplanting (DAT) to the last harvest, irrigation treatments were applied and the soil water (SW) was monitored using a Time Domain Reflectometer (TDR) HydroSense II Water Sensor with 20 cm Rods (Campbell Scientific, Inc.) previously calibrated on the trial site (Chapter 3). The soil depth of 40 cm was adopted to cover the root zone where the most effective roots are located taking into account findings on root depth of *A. cruentus* (Fasinmirin et al. 2008) and the low capacity of geotropic growth in plants after transplanting (Egel and Martyn 2008). The SW measured at 20-40 cm depth was done as reported in Chapter 3. Water application for each experimental plot was based on SW measured every two days using a handheld TDR. From the beginning of irrigation treatments to 30 DAT, the plants were only irrigated when 20.0, 16.4 and 12.7 mm of SW measured at 0-20 cm depth was achieved for 80AW, 50AW and 20AW treatments respectively; after that, 41.4, 33.5 and 25.6 mm of SW at the top 40 cm were used as the limit to irrigate. The amount of 6.2 mm of water per application was applied per plot at irrigation interval varying from one to two days, three to four and five to six days to guarantee that SW was not below the pre-defined levels of 80AW, 50AW and 20AW treatments respectively. The plants were irrigated manually using a watering can of 13 L capacity. Three rain gauges distributed in field trials were used for measuring the amount of rainfall.

The harvesting frequency treatments started at 25 DAT. The plants were harvested every two weeks (in total four harvests) or every three weeks (three harvests) over eight weeks. The less severe harvesting procedure (topped by 25% of their heights) was used in all harvests to simulate the most common practice in the area. During the first harvest, the plant was topped by 25% of main stem height and afterwards all side-shoots longer than 3 cm were topped to the same intensities. In the final harvest the entire aboveground plant was removed.

Amaranthus hybridus seeds used for these trials were collected in March 2013 from a producer field in Palma district (Northern Mozambique) and multiplied in the fields of the FAEF, and *A. tricolor* seeds were obtained through the ARC-Roodeplaat

(South Africa). The seeds germinated in seedling trays filled with Hygrotech Seedling Mix in a net-covered house and was grown in the trays for 29, 36, 29, 27, 31 and 39 days before transplanting for RS1, RS2, RS3, DS1, DS2 and DS3 respectively. Nitrogen (N) fertilizer at a rate of 14.4 kg ha^{-1} was applied using urea (46% N) at 14 and 21 days after sowing.

The soil in the trial site was a sandy soil with more than 90% sand, non-saline with less than 0.1 mS cm^{-1} of electrical conductivity and soil organic matter of about 0.50% in the top 20 cm decreasing to 0.07% at 20-40 cm depth. In the top 40 cm of soil, the permanent wilting point, field capacity and total available water were 20.2, 46.6 and 26.4 mm respectively. A rate of 35 kg ha^{-1} of each nutrient: nitrogen, phosphorus and potassium was applied in the field three days before transplanting using a mixture of urea, $NPK_{(12:24:12)}$ and potassium sulphate at a rate of 38, 146 and 35 kg ha^{-1} respectively. Nitrogen at a rate of 35 kg ha^{-1} each was applied as top dressing at 15, 30 and 45 days after transplanting (DAT) using urea, except in RS1 and DS1 seasons where only two applications (20 and 40 DAT) were done at the same rate.

The mean monthly temperature recorded at the Mavalane International Airport weather station located within a radius of 5 km from the site trials and rainfall during the experimental periods are shown in Figure 6.1.

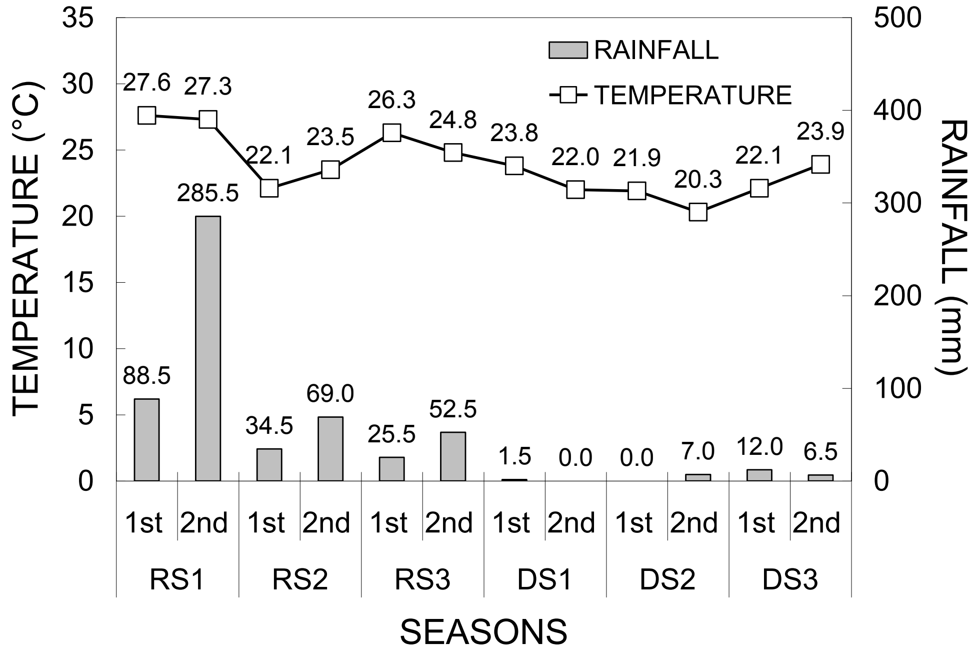

Figure 6.1: Rainfall and mean temperature recorded at first (1st) and second (2nd) months of the growing period during January-March 2014 (RS1), September-November 2014 (RS2), February-April 2015 (RS3), April-June 2014 (DS1), May-July 2015 (DS2) and August-October 2015 (DS3).

After 30 DAT, during the rainy season the plants grew with an average of SW above values of the intended watering regime (WR) and during the dry seasons around the level of the pre-defined WR (Figure 6.2). The final harvest was done at 67, 64, 64, 69, 66 and 60 DAT for RS1, RS2, RS3, DS1, DS2 and DS3 respectively.

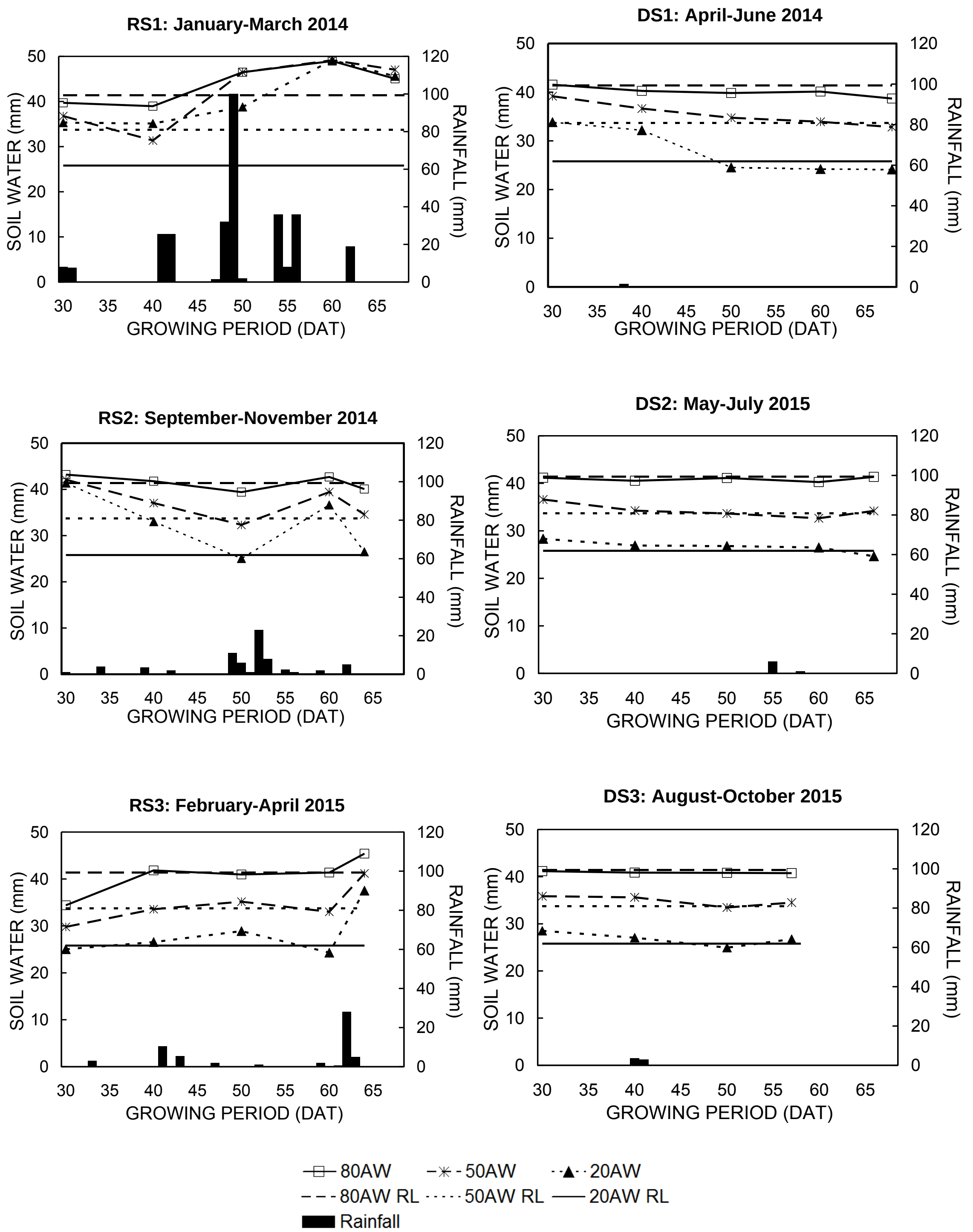

Figure 6.2: Rainfall and soil water (average of every 10 days) measured in the top 40 cm of soil during the different trial periods. The three required levels (RL) of watering regimes (WR) are shown (80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW) (Source: Chapter 5).

Data collection

Data collection was done on the four randomized central plants of each plot for all seasons to prevent border effects and all measurements were done on the aboveground plant parts. From the soil level to the youngest fully expanded apex leaf, two plant heights (H_0 and H_1) were measured at 6 and 21 DAT using a tape measure. The initial height growth was calculated by the difference between H_1 and H_0 and used as covariate. The collected leaves were counted in each harvests and the sum of the all harvests was used as final leaf number. The same procedure was used to calculate the side-shoot number from the second harvest. From the collected leaves, the length and width of seven randomly selected leaves were measured with a tape measure in each harvest and the leaf area was calculated using a linear equation (Kvet and Marshall, 1971):

Leaf area (cm^2) = 0.654 x (L x W);

Where: 0.654 = leaf shape coefficient; L = length of leaf (cm); W = width of leaf (cm) measured at half length.

The mean leaf area was used as leaf size and the total leaf area was calculated by multiplying leaf size with leaf number in each harvest. The average of the four harvests was used as the final leaf area. In each harvest, the leaves and stems (main stem, side-shoots and trace or shorter inflorescence in same cases) were dried at 80°C for 48 hours and their dry mass determined. Note that in the final harvest the removed aboveground plants were divided into leaves and stems before being dried. The final leaf and stem dry mass were calculated by the sum of all harvests. The aboveground biomass was calculated by adding the final leaf and stem dry mass. By dividing the leaf dry mass by stem dry mass, the leaf:stem (L/S) ratio was calculated. The analysis of calcium and nitrogen contents in the leaves was conducted at the Soil Laboratory of the FAEF using a composite dry leaf sample of the three trial replications per treatment as samples; it was replicated during RS1, RS2, DS1 and DS2. The samples were collected at the first and final harvest in each season. Calcium content was determined by the Complexometric Titration Method described by Waling et al. (1989) and the total nitrogen by the Kjeldahl Method. The factor 6.25 was used to convert the nitrogen to crude protein (CP).

Data analysis

The data, except calcium and crude protein content, were subjected to analysis of covariance using the MSTAT-C Version 1.2 computer program. This analysis with initial height growth as covariate was used to control experimental error and to adjust treatment means. Adjusted means were analyzed using Duncan´s New Multiple Range Test (DMRT) at the 0.05 level of probability. The data for the six seasons were analyzed separately and only the main effects of watering regimes (WR), species (S) and harvesting frequency are presented and discussed due to inconsistent results of the interaction effects. A combined analysis of covariance over transplanting date (TD) was performed with species, watering regime and season as factors for each type of season (rainy and dry) to compare diverse climatic conditions and measure the response to treatments at different TD.

For calcium and crude protein content using the same statistic computer program and DMRT to compare treatment means, a combined analysis of variance over harvesting time was performed with species, watering regime and harvesting time as factors and season as replication. This analysis was used to compare different stages of plant development and measure the response to treatments at different harvesting times.

Results and discussion

Vegetative growth

In general, leaf and side-shoot numbers, and leaf area were high at 80AW and 50AW conditions in both seasons (Table 6.1). The effects of 80AW and 50AW treatments differed only in few cases. The result at 50AW might be attributed to the reduced leaf canopy by successive harvests associated with the mechanisms developed by *A. hybridus* and *A. tricolor* to tolerate water deficit (Slabbert et al. 2004), since reduced leaf area is one of several plant adaptations to limited water availability (Anjum et al. 2011, Akinci and Lösel 2012). However, no significant difference in leaf and side-shoot numbers was observed among WR during RS1 (Table 6.1) where the SW was higher than the predefined water level most of the time (Figure 6.2). It appears that organ differentiation is more resilient to water deficit than expansion growth as also stated by Blum (2011).

As shown in Table 6.1, *A. tricolor* developed smaller leaves but both species showed similar leaf and side-shoot numbers during the rainy and dry seasons. In terms of total leaf area, it tended to be similar in both species during the rainy season but it was higher in *A. hybridus* during the dry season. It shows that *A. hybridus* is better adapted as leafy vegetables with frequent leaf harvesting during the dry season.

Table 6.1: Main effects of the water regime, amaranths species and harvesting frequency on leaf and side-shoot numbers and leaf area of amaranth leafy vegetables grown at the experimental station of Eduardo Mondlane University

Parameters	Treatments	Rainy and hot seasons			Dry and cool seasons		
		RS1	RS2	RS3	DS1	DS2	DS3
Leaves (number)	80AW	284.1	394.4**a**	334.1**a**	169.6**ab**	203.8**a**	327.7**a**
	50AW	241.4	377.7**a**	341.8**a**	179.9**a**	218.1**a**	251.6**b**
	20AW	207.3	298.4**b**	270.3**b**	140.8**b**	179.7**b**	253.7**b**
		ns	**	**	*	**	**
	A. hybridus	268.6	351.6	304.2	166.8	205.7	250.0
	A. tricolor	219.9	362.1	326.6	160.0	195.4	305.3
		ns	ns	ns	ns	ns	*
	2 weeks	271.5	389.0	343.3	169.1	203.3	302.4
	3 weeks	217.0	324.7	287.5	157.8	197.8	253.0
		*	**	**	ns	ns	*
	CV (%)	30.62	14.13	13.17	20.98	12.26	17.76
Side-shoots (number)	80AW	40.1	50.3**a**	36.8**a**	20.6	27.8**a**	39.2**a**
	50AW	33.9	50.3**a**	39.0**a**	23.3	30.7**a**	32.3**b**
	20AW	31.6	41.2**b**	30.6**b**	20.5	24.5**b**	32.3**b**
		ns	*	*	ns	**	*
	A. hybridus	37.2	47.0	36.0	21.4	29.3	32.2
	A. tricolor	33.2	47.5	34.9	21.5	26.1	37.0
		ns	ns	ns	ns	*	ns
	2 weeks	42.5	54.7	41.3	23.5	29.3	39.3
	3 weeks	27.9	39.8	29.6	19.4	26.1	29.9
		**	**	**	*	*	**
	CV (%)	26.61	15.54	17.75	21.88	12.39	16.92
Leaf size (cm^2)	80AW	30.3**a**	22.9**a**	25.6**a**	15.5**ab**	15.2**a**	13.5**a**
	50AW	30.6**a**	22.4**a**	24.9**ab**	16.9**a**	14.7**a**	11.0**b**
	20AW	24.8**b**	16.9**b**	21.9**b**	13.7**b**	11.2**b**	11.4**b**
		*	**	*	*	**	**
	A. hybridus	31.9	22.0	25.3	16.6	14.6	12.2
	A. tricolor	25.2	19.4	22.9	14.1	12.8	11.7
		**	*	*	*	*	ns
	2 weeks	25.8	19.3	22.4	13.7	12.2	11.1
	3 weeks	31.3	22.2	25.9	17.0	15.3	12.8
		**	*	**	**	**	**
	CV (%)	16.67	14.98	13.89	18.24	17.20	11.12
Total leaf area (cm^2 $plant^{-1}$)	80AW	8677**a**	9096**a**	8542**a**	3117**a**	3457**a**	4516**a**
	50AW	7904**ab**	8456**a**	8407**a**	3428**a**	3400**a**	2746**b**
	20AW	5167**b**	5269**b**	5932**b**	1985**b**	2069**b**	3050**b**
		*	**	**	**	**	**
	A. hybridus	8859	8036	7778	3188	3263	3238
	A. tricolor	5639	7178	7476	2498	2688	3637
		**	ns	ns	*	*	ns
	2 weeks	7468	7748	7744	2564	2672	3550
	3 weeks	7031	7466	7510	3123	3279	3325
		ns	ns	ns	ns	*	ns
	CV (%)	40.78	26.06	20.21	35.11	24.74	25.21

In each section, adjusted means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; CV, coefficient of variation; 80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW; 2 weeks, harvested every two weeks; 3 weeks, harvested every three weeks; RS1, January-March 2014; RS2, September-November 2014; RS3, February-April 2015; DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015.

During the rainy season, plants harvested fortnightly produced more side-shoots as well as more and smaller leaves (Table 6.1); however, the total leaf area did not differ from those obtained at 3 weeks interval. Similar results for side-shoot number and leaf area were also found during the dry season while the leaf number was not affected by harvesting frequency except in DS3. Since peak light intensities occur during the rainy season in the trial region, the observed difference in leaf number (Table 6.1) was probably due to larger exposure of plants harvested every 2 weeks to high light intensities (Khandaker et al. 2009). With regard to side-shoots, the higher number obtained in plants harvested four times (every two weeks) compared to plants harvested three times (3 weeks interval) was due to removal of apical dominance in young shoot tips which promote the emergence of lateral buds (Cline 1997). Norman and Shongwe (1993) also found an increase in side-shoot number in plants harvested at 2 weeks interval in *A. hybridus*. In terms of leaf production, these results suggest that both harvesting frequencies can be done during rainy and dry seasons, since there is no significant difference in total leaf area in most of the trial seasons. As the consumer prefers to eat smaller and tender green leaves, the fortnightly harvest is recommended for both seasons.

Aboveground yield components

As shown in Table 6.2, the highest aboveground biomass, leaf and stem yields were obtained at 80AW and 50AW conditions during most of the trial seasons (rainy and dry). During the dry season, the increased aboveground biomass, leaf and stem yields were consistent with increased total leaf area (Table 6.1). Note that in plants harvested once and submitted to similar climatic conditions and soil characteristics, the highest aboveground biomass and leaf yield were obtained at 80AW conditions during the dry season as reported in Chapter 3.

Table 6.2: Main effects of the water regime, amaranths species and harvesting frequency on aboveground yield components and leaf:stem (L/S) ratio of amaranth leafy vegetables grown at the experimental station of Eduardo Mondlane University

Parameters On dry mass basis	Treatments	Rainy and hot seasons RS1	 RS2	 RS3	Dry and cool seasons DS1	 DS2	 DS3
Aboveground biomass	80AW	54.66**a**	49.16**a**	31.20**a**	9.86**a**	12.71**a**	17.80**a**
(g plant^{-1})	50AW	53.36**a**	40.07**a**	26.59**b**	10.17**a**	11.34**a**	10.72**b**
	20AW	36.72**b**	26.27**b**	18.13**c**	7.13**b**	8.00**b**	11.10**b**
		*	**	**	*	**	**
	A. hybridus	55.76	41.99	26.82	9.97	11.90	12.43
	A. tricolor	40.74	35.00	23.79	8.14	9.47	13.98
		**	ns	ns	ns	*	ns
	2 weeks	45.23	35.43	24.22	9.18	10.60	12.72
	3 weeks	51.27	41.56	26.39	8.93	10.76	13.69
		ns	ns	ns	ns	ns	ns
	CV (%)	27.77	34.54	18.55	30.26	25.99	24.14
Leaves	80AW	24.35**a**	21.49**a**	17.52**a**	5.70**a**	7.01**a**	8.82**a**
(g plant^{-1})	50AW	23.04**a**	16.52**b**	15.54**a**	6.05**a**	6.62**a**	5.86**b**
	20AW	16.74**b**	11.21**c**	11.13**b**	4.24**b**	4.86**b**	5.84**b**
		*	**	**	*	**	**
	A. hybridus	25.14	18.30	15.62	5.92	6.88	6.40
	A. tricolor	17.62	14.51	13.85	4.73	5.44	7.28
		**	*	*	*	**	ns
	2 weeks	21.55	16.35	15.01	5.47	6.03	7.11
	3 weeks	21.21	16.46	14.46	5.19	6.30	6.57
		ns	ns	ns	ns	ns	ns
	CV (%)	27.79	30.50	15.91	30.34	20.68	21.14
Stems	80AW	22.34**b**	27.67**a**	13.68**a**	4.16**a**	5.70**a**	8.98**a**
(g plant^{-1})	50AW	30.32**a**	23.54**a**	11.05**b**	4.12**a**	4.72**a**	4.87**b**
	20AW	19.98**b**	15.06**b**	6.99**c**	2.89**b**	3.14**b**	5.26**b**
		**	**	**	*	**	**
	A. hybridus	30.62	23.69	11.20	4.04	5.02	6.04
	A. tricolor	23.12	20.49	9.95	3.41	4.03	6.70
		*	ns	ns	ns	ns	ns
	2 weeks	23.67	19.08	9.22	3.71	4.58	5.61
	3 weeks	30.06	25.10	11.93	3.75	4.47	7.13
		*	*	**	ns	ns	*
	CV (%)	28.80	38.71	25.54	33.03	34.50	29.28
L/S ratio	80AW	0.85	0.84	1.38**b**	1.61	1.54	1.04**b**
	50AW	0.79	0.77	1.50**b**	1.70	1.53	1.20**a**
	20AW	0.84	0.84	1.72**a**	1.64	1.66	1.20**a**
		ns	ns	**	ns	ns	*
	A. hybridus	0.87	0.87	1.58	1.68	1.59	1.15
	A. tricolor	0.79	0.77	1.49	1.62	1.57	1.14
		*	*	ns	ns	ns	ns
	2 weeks	0.92	0.93	1.75	1.67	1.51	1.29
	3 weeks	0.74	0.71	1.32	1.63	1.65	1.00
		**	**	**	ns	ns	**
	CV (%)	9.61	14.96	12.36	12.25	18.07	10.97

In each section, adjusted means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; CV, coefficient of variation; 80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW; 2 weeks, harvested every two weeks; 3 weeks, harvested every three weeks; RS1, January-March 2014; RS2, September-November 2014; RS3, February-April 2015; DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015.

It appears as if the multiple harvests reduced the drought effect on leaf dry mass as reported by Diwani and Janssens (2001) in *A. cruentus*. The L/S ratio was not affected by WR in most of the trial seasons. However, during RS3 and DS3, L/S ratio was high at the 20AW treatment. Since L/S ratio is an important factor affecting leaf quality in term of nutrient contents and using L/S ratio as an index of quality, the results showed a high leaf quality of *A. hybridus* and *A. tricolor* grown under different climatic conditions over the year in southern Mozambique, since the leaves are the edible part of the plants.

Aboveground biomass and stem yield were similar in both species during most of the trial seasons (Table 6.2). However, the highest leaf yield was produced by *A. hybridus* during rainy and dry seasons, except in DS3. With regard to L/S ratio, *A. hybridus* showed a higher leaf quality during the rainy season but similar to *A. tricolor* during the dry season. These results indicate that *A. hybridus* as a leafy vegetable exhibited better performance all year round, particularly evident during the rainy season.

No significant differences were found in aboveground biomass and leaf yield between the two harvesting frequencies during the rainy and dry seasons. Stem yield was higher when the plants were harvested every 3 weeks, while the highest L/S ratio was found in plants harvested fortnightly during the rainy season. The high stem yield obtained in plants harvested at 3 weeks interval was due to accumulation of carbohydrate reserves in the stem. Mobilization of these carbohydrate reserves to the buds for re-growth of new leaves (Vargas-Ortiz et al. 2013) occurs in plants harvested every 2 weeks as indicated by the high L/S ratio.

As shown in Table 6.3, the effect of interactions did not affect leaf yield and also there is no significant difference between TD during the rainy seasons. The highest leaf yields were found in plants grown at 80AW and 50AW conditions (18.36 and 20.54 g plant^{-1}) and in *A. hybridus* (19.62 g plant^{-1}) harvested at 2 and 3 weeks intervals. Under the conditions experienced during the rainy season, the one week difference between the two harvesting frequencies did not result in a significant change in leaf yield of both species as it was found in *A. hybridus* by Norman and Shongwe (1993). Note that the high rainfall amount received probably alleviated the adverse effect of drought stress on leaf yield, mainly during RS1 and RS2 (Figure 6.2).

Table 6.3: Combined analysis of covariance over transplanting date on leaf yield during rainy and dry seasons

Source	df	RAINY SEASON		DRY SEASON	
		Probability	Leaf yield (g plant^{-1})	Probability	Leaf yield (g plant^{-1})
Transplanting date (TD)	2	ns		0.0000**	
Repetitions within TD	6	0.1263^{ns}		0.0000**	
Watering regime (WR)	2	0.0000**		0.0000**	
80AW			20.54**a**		
50AW			18.36**a**		
20AW			13.61**b**		
TD x WR	4	0.3574^{ns}		0.0022**	#
Species (S)	1	0.0001**		0.0137*	
A. hybridus			19.62		
A. tricolor			15.39		
TD x S	2	0.2011^{ns}		0.0209*	#
WR x S	2	0.2936^{ns}		ns	
TD x WR x S	4	0.0710^{ns}		0.1812^{ns}	
Harvesting frequency (F)	1	ns		ns	
TD x F	2	ns		ns	
WR x F	2	0.3166^{ns}		ns	
TD x WR x F	4	ns		ns	
S x F	1	ns		0.1998^{ns}	
TD x S x F	2	ns		ns	
WR x S x F	2	ns		ns	
TD x WR x S x F	4	ns		0.0804^{ns}	
Covariate	1				
Error	65				
Total	107				
Coefficient of variation			28.04%		23.77%

Mean comparisons are presented in Figure 6.3.
In each section, adjusted means followed by the same letters in the column are not significantly different at 5% level probability (DMRT); ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively; 80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW.

A significant interaction with regard to leaf yield was shown between TD and WR during the dry season (Table 6.3). As shown in Figure 6.3A, leaf yield decreased with a decrease in SW from 80% to 50% of AW in DS3 and from 50% to 20% of AW in DS1. In DS2, the leaf yield was higher at 80AW compared to 20AW conditions.

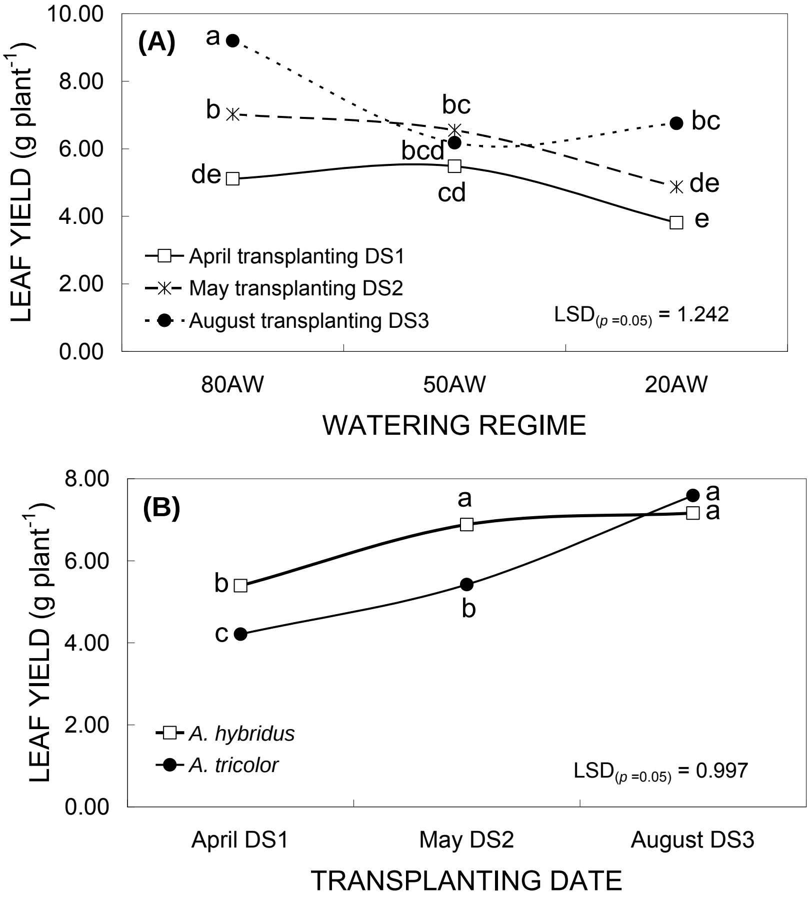

Figure 6.3: Interaction between (**A**) watering regime (80AW, irrigated at 80% of total available water (TAW); 50AW, irrigated at 50% of TAW; 20AW, irrigated at 20% of TAW) and transplanting date and (**B**) transplanting date and species affecting the leaf yield during dry season (DS1, April-June 2014; DS2, May-July 2015; DS3, August-October 2015). Means followed by the same letters in each figure are not significantly different at 5% level probability (DMRT).

The highest leaf yield (9.20 g plant^{-1}) was obtained at 80AW conditions in DS3 (Figure 6.3A). For plants growing in the same growth season and under similar soil characteristics but harvested once, the leaf yield was higher in DS1 than in DS3 (Chapter 3). This different result in this study was probably due to multiple harvests which prolonged the vegetative growth phase (Palada and Chang 2003, Awe and Osunlola 2013). It is a great advantage for amaranth production as a leafy vegetable, since short days induce early flowering in *A. hybridus* and *A. tricolor* during the dry season in the south of Mozambique (Chapter 3). Furthermore, the leaf yield was also affected by an interaction between TD and S (Table 6.3). Both species increased leaf yield with transplanting made from April to August with the highest leaf yield observed in *A. hybridus* (5.39 and 6.88 g plant^{-1}) compared to *A. tricolor* (4.21 and 5.42 g plant^{-1}) during the DS1 and DS2 seasons (Figure 6.3B). It indicates that *A. hybridus* was better adapted to frequent leaf harvests (every 2 or 3 weeks) during the beginning and the middle of the dry season. However, no significant difference was found in leaf yield of both species (7.16 and 7.59 g plant^{-1}) during the DS3 season.

From Figure 6.3A and B, it is clear that the highest leaf yield was obtained with transplanting in August. Since the temperature greatly affected the leaf yield in these two species (Chapter 3), the higher leaf yield obtained in DS3 compared to DS1 might be explained by an increase in temperature from 1st to 2nd months of growing period during DS3 and vice-versa in DS1 (Figure 6.1). However, the difference in leaf yield during DS1 and DS2 could be due to other factors besides temperature.

The minimum daily consumption recommended by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) is 400 g person^{-1} of fresh weight of fruit and vegetables (WHO/FAO 2003) which corresponds to 80 g day^{-1} person^{-1} of dry mass assuming the ratio of 20% of dry mass for those categories of food (FAO 1995). Each plant of *A. hybridus* produced an average of 6 g in 4 harvests, equivalent to 1.5 g per harvest, and the interval between harvests is 15 days which gives 0.1 g day^{-1}. Assuming that the amaranth leaves can contribute 25% of 80 g day^{-1} person^{-1}, 200 plants per person is required to achieve 20 g day^{-1} of the dry mass recommended. It means in actual terms the use of one area of 87.5 m^2 per family every two months taking into account: 200 plants are required for 15 days which correspond to an area of 17.5 m^2 per person, plants spaced at 25 x 35 cm, and a family size of 5 persons (Mather et al. 2008). In the following two months, an equal area will be required totalizing thus 175 m^2 for the first four months of the dry season. The former area can be used for the remaining two months of the season. Note that during the rainy season the area needed is smaller due to the seasonal high leaf yield. As the average area per family is about 1.63 ha in rural areas (Mather et al. 2008) only 1.07% of the total area would be occupied with amaranth species which may be appropriated to the crop system.

The results from this study showed that *A. tricolor* as a leafy vegetable was less adapted to frequent harvests (every 2 or 3 weeks) under different WR during rainy and dry seasons. It is in line with Grubben (2004) who stated that multiple harvests in *A. tricolor* are less suitable compared to other amaranth species.

Calcium and crude protein contents in the leaves

The calcium content in leaves was only affected by harvesting time. It was high in both species (2529 mg 100 g^{-1}) at final harvest (Figure 6.4A) but much lower than those reported in *A. tricolor* and *A. hybridus* (3448-5675 mg 100 g^{-1}) harvested once (Chapter 3).

The harvesting time also affected crude protein (CP) content in leaves of both species. In this case, CP decreased from 17.0% at first to 14.5% at final harvests

(Figure 6.3B). The CP content was also different between the species. The highest value of 16.5% CP was obtained in *A. tricolor* leaves while the lowest of 14.9% CP was obtained in *A. hybridus* (Figure 6.4C).

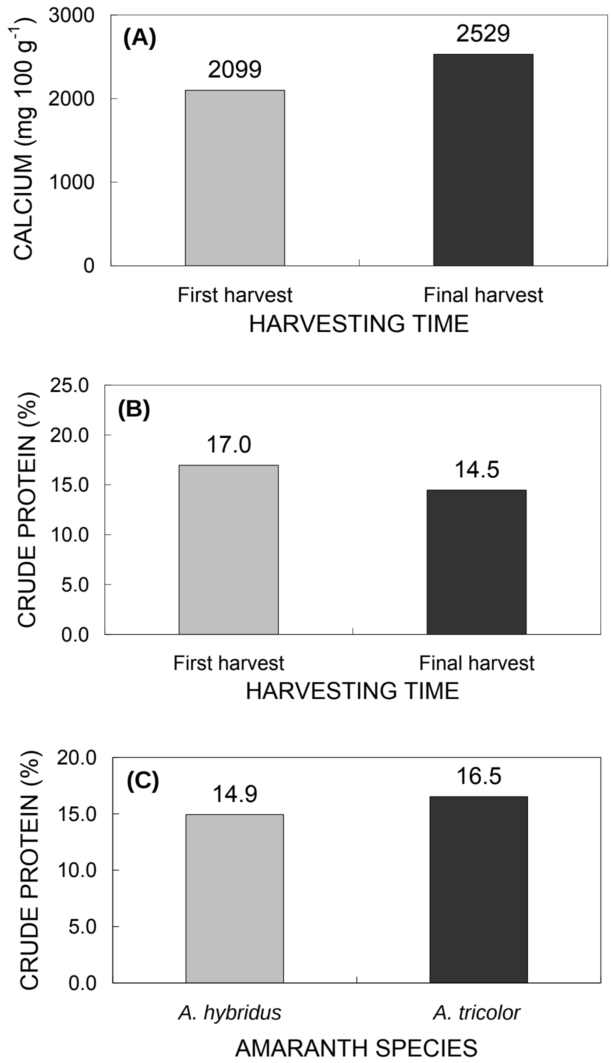

Figure 6.4: Leaf calcium (**A**) and crude protein content (**B**) affected by harvesting time, and crude protein content in *A. hybridus* and *A. tricolor* (**C**) under different watering regimes and harvesting frequency.

These two values were higher than those reported in *A. tricolor* (14.92%) and *A. hybridus* (12.94%) harvested once (Chapter 3) but lower compared to 17.92% in *A. hybridus* (Akubugwo et al. 2007) and 28.23% in *Spinacia oleracea* (Rao et al. 2015).

Although the crude protein values found in this study were lower than the spinach (*Spinacia oleracea*), Mnkeni et al. (2007) stated that the protein content in five amaranth accessions are comparable to amount found in spinach and more higher than in cabbage. Under hot conditions, Modi (2007) found similar values around 15-16% CP in *A. hybridus* and *A. tricolor*. These findings confirms the influence of plant age on calcium and crude protein content in leaves of these two amaranth species as also reported by Modi (2007). Moreover, their leaves can be used as a supplement of calcium and protein in the diet of people of southern Mozambique, without nutritive changes caused by water deficit and harvesting frequency.

Conclusions

This study reveals that *A. hybridus* and *A. tricolor* were tolerant to water deficit at 50% of total available water with regard to vegetative growth when submitted to frequent leaf harvests during rainy and dry seasons. However, the highest leaf yield was obtained from *A. hybridus* under the different watering regimes with successive harvests at 2 or 3 weeks intervals throught the year. The harvesting frequency did not affect aboveground biomass and leaf yield. The leaf calcium content increased and the crude protein decreased with the age of the plant. The crude protein in leaves of *A. tricolor* was higher than in *A. hybridus*.

Under the conditions experienced during these trials, this study fulfilled the issue related to the influence of harvesting frequency on amaranth leafy vegetable under water deficit and provides relevant information to plan a suitable harvesting regime. It proved that *A. hybridus* as leafy vegetable and source of calcium and protein is better adapted to the climatic conditions with harvests at two-week intervals since this frequency yielded younger, small and tender green leaves which are the consumer preference. In addition, this species occurs naturally in Mozambique and when it is cultivated in a small area around 100 m^2 may provide on daily basis sufficient leaves for a family of five every two months during the dry season. It is evident that *A. hybridus* if cultivated as a crop will improve food security and nutrition in southern Mozambique.

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Chapter 7

Summary discussions, conclusions and recommendations

7.1 Summary discussions

This study aimed to assess the effect of harvesting procedures and watering regimes on growth, yield and quality of *A. hybridus and A. tricolor* in southern Mozambique. In order to address the objectives presented in the Introduction, three field experiments repeated six times in 2014 and 2015 (three during the rainy season and three in the dry season) were carried out on *A. hybridus* and *A. tricolor*, with three watering regimes (irrigated at 80%, 50% and 20% of total available water) in Maputo, Mozambique.

The objective of Experiment one was to understand how these two species respond to different soil water levels in diverse climatic conditions in term of vegetative and reproductive growth and leaf and grain nutritional contents. Results show that the interaction effect between watering regimes and species were inconsistent on vegetative and reproductive growth of both species over the year. Plants of both species that were harvested once at maturity stage did not tolerate water deficit at 50% and 20% of total available water. However, the leaf number, leaf area and leaf yield of both species were less susceptible to intermittent drought stress that occurred in intervals of sporadic rainfall during the rainy season. The time to flowering and the grain crude protein contents were unaffected by the water deficit. The calcium and crude protein content in the leaves improved at lower levels of soil water (50% and 20% of total available water) with highest values obtained in *A. tricolor*. This fact indicates that both species have the aptitude to be a source of proteins throughout the year with values ranging from 12.89% to 14.16%. The high leaf yield was greatly affected by temperature during the rainy season with increased day lengths. However, during the dry season the low leaf yield was determined by the length of the day and it decreased with short day lengths. Both species had similar behavior in relation to different soil water and climate conditions during the rainy and dry seasons in southern Mozambique. The onset of flowering was determined by the day length and the minimum temperature with day length the most determining factor. The increase in minimum temperature delayed flowering mainly when the day length was above 12 hours day^{-1}. Under these conditions, the highest grain yield and harvest index was found in *A. tricolor* plants.

Experiments two and three aimed to understand how both species respond to the same abovementioned soil water levels when submitted to multiple leaf harvests under different intensities (experiment two) and frequencies (experiment three) in term of vegetative growth and leaf nutritional contents. An additional aim was to determine what harvest procedures (intensity and frequency) are more suitable for those species under these conditions. The results revealed insufficient evidence to support the hypothesis that the combined effects of watering regimes and harvesting intensity, and watering regimes and harvesting frequency affect vegetative growth of *A. hybridus* and *A. tricolor*. However, the leaf and side-shoot numbers, leaf area and leaf yield of both species were tolerant to water deficit at 50% of total available water. In relation to harvesting intensity and frequency, it turned out that *A. hybridus* is better adapted to the climatic conditions in plants topped to 25% of their heights with harvests at two-week intervals over the year. This frequency yielded more small and tender green leaves which are the preference of consumers. The great advantage found was that multiple leaf and side-shoot harvests extended the vegetative growth phase, especially under short days during the dry season. In plants harvested several times, the calcium and crude protein content in the leaves were unaffected by watering regimes but was affected by plant age. In both species, the leaf calcium content increased from interval of 1730-2099 mg 100 g^{-1} (first harvest) to 2529-2724 mg 100 g^{-1} (last harvest). On the contrary, the crude protein decreased from 17.0-18.3% to 12.7-14.5%.

This study revealed that *A. hybridus* as leafy vegetable have potential to be cultivated under rain-fed condition with supplemental irrigation during the rainy season. However, during the dry season, it might be cultivated as an irrigated crop at 50% of total available water. It can be cultivated in a small area less than 100 m^2 to provide sufficient leaves for a family of five on daily basis every two months during the rainy and dry season. Moreover, this species occurs naturally in Mozambique consequently ecologically adapted to southern Mozambique and the best harvesting intensity (topped to 25%) found is similar to the intensity used by local people. It might be a good indication that it will be easily adopted by smallholder farmers.

Amaranthus tricolor as a grain crop have the potential to be cultivated from October to February while during other months of the year, both species showed the viability to be cultivated if irrigation is applied to ensure a soil water content of 80% of total available water. Althougth amaranth grain is not used as a staple food, these amaranth species can be a potential crop to be promoted in order to diversify the diet of people in Mozambique.

It is clear that *Amaranthus hybridus*, as a leafy vegetable, shows the potential to be a suitable crop all year round and a supplement of calcium and protein in the diet of rural communities.

7.2 Conclusions

The response of *A. hybridus* and *A. tricolor* to different watering regimes revealed that the vegetative growth and leaf yield were reduced at 50% and 20% of total available water in plants harvested once at maturity stage. However, the leaf yield was less sensitive to short interval water deficits during the rainy season. Calcium and crude protein content in the leaves improved at lower levels of soil water, 50% and 20% of total available water with highest values obtained in *A. tricolor*. Both species had similar behavior in relation to different soil water and climate conditions during the rainy and dry seasons in southern Mozambique. The temperature greatly affected the maximum leaf yield of both species during the rainy season with increased day lengths, while the low yield was determined by the length of the day during the dry season under short day lengths.

Grain yield was also reduced at 50% and 20% of total available water in plants of both species harvested once at maturity stage. However, time to flowering and the grain crude protein contents were unaffected by the water deficit. The day length was the most determinant factor on the onset of flowering. The highest grain yield was produced by *A. tricolor* when day lengths were above 12 hours day^{-1}.

Vegetative growth and leaf yield of *A. hybridus* and *A. tricolor* decreased only at 20% of total available water when submitted to multiple leaf harvests (plants topped by 25% and 50% of their heights). The leaf calcium content increased and the crude protein decreased with the age of the plant. High leaf yield was achieved when the *A. hybridus* plants were topped by 25% of their height over the year.

Vegetative growth and leaf yield of both species decreased only at 20% of total available water when submitted to frequent leaf harvests (2 or 3 weeks intervals) during rainy and dry seasons. The highest leaf yield was obtained by *A. hybridus* under the different watering regimes with successive harvests at 2 or 3 weeks intervals around the year. However, the two-week intervals frequency yielded younger, small and tender green leaves which are the consumer preference. The leaf calcium content increased and the crude protein decreased with the age of the plant.

7.3 Recommendations

Amaranthus hybridus is recommended to be cultivated as leafy vegetable during:

- the rainy season under rain-fed condition with supplemental irrigation;
- the dry season as an irrigated crop at 50% of total available water;
- in both seasons, the leaf harvesting should be topped by 25% at 2 weeks intervals.

7.4 Suggestion for further research

Further studies are needed in order to make these conclusions applicable. It is suggested evaluating the:

1. Results of this study on-farm;
2. Influence of combined effect between harvesting intensity and frequency on vegetative growth and leaf nutritional content of *A. hybridus* under different watering regimes during the rainy and dry seasons in southern Mozambique;
3. Response of *A. hybridus* and *A. tricolor* at photoperiod below 11 hours day^{-1} (May and June sowing) in term of grain yield.

Appendices

A1: Results of analysis of variance (ANOVA) for Chapter 3.

A1.1: ANOVAs for height growth (cm)

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	11480.056	2296.011	9.7206	0.0001**
		Watering regime	2	1752.287	876.143	3.7093	0.0388*
		Species	1	780.271	780.271	3.3034	0.0811^{ns}
		WR x S	2	344.136	172.068	0.7285	F<1^{ns}
		Error	25	5905.021	236.201		
		Total	35	20261.770			
		CV = 9.59%					
	September-November 2014	Repetitions (blocks)	5	8063.794	1612.759	17.2733	0.0001**
		Watering regime	2	1019.272	509.636	5.4584	0.0108**
		Species	1	99.301	99.301	1.0636	0.3123^{ns}
		WR x S	2	582.484	291.242	3.1193	0.0617^{ns}
		Error	25	2334.181	93.367		
		Total	35	12099.032			
		CV = 15.03%					
	February-April 2015	Repetitions (blocks)	5	504.305	100.861	0.5925	F<1^{ns}
		Watering regime	2	2711.400	1355.700	7.9644	0.0021**
		Species	1	572.805	572.805	3.3651	0.0785^{ns}
		WR x S	2	490.324	245.162	1.4403	0.2559^{ns}
		Error	25	4255.492	170.220		
		Total	35	8534.326			
		CV = 14.44%					
Dry	April-June 2014	Repetitions (blocks)	5	4299.541	859.908	13.9476	0.0001**
		Watering regime	2	1584.559	792.279	12.8507	0.0001**
		Species	1	174.240	174.240	2.8262	0.1052^{ns}
		WR x S	2	702.579	351.289	5.6979	0.0091**
		Error	25	1541.319	61.653		
		Total	35	8302.237			
		CV = 13.47%					
	May-July 2015	Repetitions (blocks)	5	1665.852	333.170	6.5027	0.0005**
		Watering regime	2	6341.531	3170.766	61.8859	0.0001**
		Species	1	32.680	32.680	0.6378	F<1^{ns}
		WR x S	2	13.351	6.675	0.1303	F<1^{ns}
		Error	25	1280.892	51.236		
		Total	35	9334.307			
		CV = 13.91%					
	August-October 2015	Repetitions (blocks)	5	353.028	70.606	3.7119	0.0119*
		Watering regime	2	4210.242	2105.121	110.670 8	0.0001**
		Species	1	21.314	21.314	1.1205	0.2999^{ns}
		WR x S	2	30.162	15.081	0.7928	F<1^{ns}
		Error	25	475.537	19.021		
		Total	35	5090.283			
		CV = 10.14%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A1.2: ANOVAs for internode length (cm)

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	2.650	0.530	4.8654	0.0030**
		Watering regime	2	0.952	0.476	4.3681	0.0236*
		Species	1	1.604	1.604	14.7287	0.0008**
		WR x S	2	0.071	0.035	0.3238	F<1^ns
		Error	25	2.723	0.109		
		Total	35	8.000			
		CV = 8.18%					
	September-November 2014	Repetitions (blocks)	5	11.231	2.246	10.0876	0.0001**
		Watering regime	2	1.847	0.924	4.1477	0.0278*
		Species	1	0.967	0.967	4.3423	0.0476*
		WR x S	2	0.817	0.409	1.8350	0.1805^ns
		Error	25	5.567	0.223		
		Total	35	20.430			
		CV = 18.53%					
	February-April 2015	Repetitions (blocks)	5	0.463	0.093	1.2172	0.3302^ns
		Watering regime	2	0.645	0.323	4.2360	0.0260*
		Species	1	0.934	0.934	12.2738	0.0018**
		WR x S	2	0.004	0.002	0.0255	F<1^ns
		Error	25	1.903	0.076		
		Total	35	3.950			
		CV = 8.85%					
Dry	April-June 2014	Repetitions (blocks)	5	5.242	1.048	11.2059	0.0001**
		Watering regime	2	1.702	0.851	9.0933	0.0011**
		Species	1	0.422	0.422	4.5155	0.0437*
		WR x S	2	0.802	0.401	4.2839	0.0251*
		Error	25	2.339	0.094		
		Total	35	10.507			
		CV = 13.85%					
	May-July 2015	Repetitions (blocks)	5	0.823	0.165	3.5185	0.0152*
		Watering regime	2	5.765	2.883	61.5919	0.0001**
		Species	1	0.538	0.538	11.4910	0.0023**
		WR x S	2	0.354	0.177	3.7809	0.0368*
		Error	25	1.170	0.047		
		Total	35	8.650			
		CV = 9.91%					
	August-October 2015	Repetitions (blocks)	5	0.318	0.064	1.2753	0.3056^ns
		Watering regime	2	5.437	2.719	54.5055	0.0001**
		Species	1	0.267	0.267	5.3520	0.0292*
		WR x S	2	0.027	0.014	0.2729	F<1^ns
		Error	25	1.247	0.050		
		Total	35	7.296			
		CV = 10.48%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A1.3: ANOVAs for leaf number

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	14898.320	2979.664	2.3727	0.0682[ns]
		Watering regime	2	63.585	31.793	0.0253	F<1[ns]
		Species	1	6955.559	6955.559	5.5386	0.0268*
		WR x S	2	60.545	30.273	0.0241	F<1[ns]
		Error	25	31395.898	1255.836		
		Total	35	53373.907			
		CV = 20.18%					
	September-November 2014	Repetitions (blocks)	5	16947.676	3389.535	17.0383	0.0001**
		Watering regime	2	284.712	142.356	0.7156	F<1[ns]
		Species	1	1386.321	1386.321	6.9687	0.0141*
		WR x S	2	193.624	96.812	0.4866	F<1[ns]
		Error	25	4973.417	198.937		
		Total	35	23785.750			
		CV = 14.46%					
	February-April 2015	Repetitions (blocks)	5	10626.941	2125.388	6.6512	0.0005**
		Watering regime	2	1330.971	665.485	2.0826	0.1457[ns]
		Species	1	8636.605	8636.605	27.0273	0.0001**
		WR x S	2	2481.954	1240.977	3.8835	0.0340*
		Error	25	7988.764	319.551		
		Total	35	31065.235			
		CV = 9.95%					
Dry	April-June 2014	Repetitions (blocks)	5	7096.629	1419.326	3.5886	0.0139*
		Watering regime	2	5566.857	2783.429	7.0376	0.0038**
		Species	1	1895.151	1895.151	4.7917	0.0381*
		WR x S	2	1030.271	515.135	1.3025	0.2897[ns]
		Error	25	9887.724	395.509		
		Total	35	25476.633			
		CV = 20.52%					
	May-July 2015	Repetitions (blocks)	5	1453.039	290.608	1.2680	0.3086[ns]
		Watering regime	2	11845.878	5922.939	25.8444	0.0001**
		Species	1	7095.254	7095.254	30.9597	0.0001**
		WR x S	2	581.607	290.804	1.2689	0.2986[ns]
		Error	25	5729.428	229.177		
		Total	35	26705.206			
		CV = 15.78%					
	August-October 2015	Repetitions (blocks)	5	709.226	141.845	0.5745	F<1[ns]
		Watering regime	2	6216.629	3108.314	12.5890	0.0002**
		Species	1	2666.001	2666.001	10.7976	0.0030**
		WR x S	2	733.869	366.935	1.4861	0.2456[ns]
		Error	25	6172.665	246.907		
		Total	35	16498.389			
		CV = 19.58%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A1.4: ANOVAs for side-shoot number

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	17.526	3.505	0.5632	F<1^{ns}
		Watering regime	2	3.691	1.845	0.2965	F<1^{ns}
		Species	1	163.840	163.840	26.3277	0.0001**
		WR x S	2	5.505	2.752	0.4423	F<1^{ns}
		Error	25	155.578	6.223		
		Total	35	346.139			
		CV = 8.40%					
	September-November 2014	Repetitions (blocks)	5	54.418	10.884	10.3351	0.0001**
		Watering regime	2	4.442	2.221	2.1092	0.1424^{ns}
		Species	1	16.947	16.947	16.0928	0.0005**
		WR x S	2	6.042	3.021	2.8688	0.0756^{ns}
		Error	25	26.327	1.053		
		Total	35	108.176			
		CV = 6.70%					
	February-April 2015	Repetitions (blocks)	5	37.625	7.525	1.8365	0.1421^{ns}
		Watering regime	2	26.097	13.049	3.1847	0.0586^{ns}
		Species	1	74.247	74.247	18.1207	0.0003**
		WR x S	2	4.407	2.204	0.5378	F<1^{ns}
		Error	25	102.434	4.097		
		Total	35	244.810			
		CV = 7.39%					
Dry	April-June 2014	Repetitions (blocks)	5	42.276	8.455	3.4266	0.0171*
		Watering regime	2	30.327	15.164	6.1453	0.0067**
		Species	1	11.560	11.560	4.6849	0.0402*
		WR x S	2	7.522	3.761	1.5241	0.2374^{ns}
		Error	25	61.688	2.468		
		Total	35	153.372			
		CV = 10.45%					
	May-July 2015	Repetitions (blocks)	5	31.768	6.354	2.6002	0.0502*
		Watering regime	2	62.911	31.455	12.8732	0.0001**
		Species	1	79.507	79.507	32.5384	0.0001**
		WR x S	2	4.671	2.335	0.9557	F<1^{ns}
		Error	25	61.087	2.443		
		Total	35	239.943			
		CV = 8.79%					
	August-October 2015	Repetitions (blocks)	5	25.055	5.011	1.4676	0.2357^{ns}
		Watering regime	2	118.302	59.151	17.3246	0.0001**
		Species	1	18.923	18.923	5.5422	0.0267*
		WR x S	2	0.527	0.263	0.0771	F<1^{ns}
		Error	25	85.357	3.414		
		Total	35	248.163			
		CV = 13.28%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A1.5: ANOVAs for leaf size (cm^2)

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	4502.352	900.470	7.3218	0.0002**
		Watering regime	2	301.984	150.992	1.2277	0.3100^{ns}
		Species	1	942.490	942.490	7.6634	0.0105**
		WR x S	2	586.512	293.256	2.3845	0.1128^{ns}
		Error	25	3074.631	122.985		
		Total	35	9407.969			
		CV = 14.19%					
	September-November 2014	Repetitions (blocks)	5	3823.388	764.678	14.5188	0.0001**
		Watering regime	2	129.044	64.522	1.2251	0.3108^{ns}
		Species	1	319.814	319.814	6.0722	0.0210*
		WR x S	2	344.461	172.230	3.2701	0.0548^{ns}
		Error	25	1316.704	52.668		
		Total	35	5933.410			
		CV = 27.39%					
	February-April 2015	Repetitions (blocks)	5	102.022	20.404	0.9227	F<1^{ns}
		Watering regime	2	132.071	66.035	2.9863	0.0687^{ns}
		Species	1	136.111	136.111	6.1553	0.0202*
		WR x S	2	52.561	26.280	1.1885	0.3213^{ns}
		Error	25	552.821	22.113		
		Total	35	975.586			
		CV = 11.46%					
Dry	April-June 2014	Repetitions (blocks)	5	2208.336	441.667	7.7040	0.0002**
		Watering regime	2	832.805	416.403	7.2633	0.0033**
		Species	1	676.867	676.867	11.8066	0.0021**
		WR x S	2	353.004	176.502	3.0787	0.0638^{ns}
		Error	25	1433.236	57.329		
		Total	35	5504.248			
		CV = 29.47%					
	May-July 2015	Repetitions (blocks)	5	500.336	100.067	4.8520	0.0031**
		Watering regime	2	1472.184	736.092	35.6912	0.0001**
		Species	1	96.040	96.040	4.6567	0.0407*
		WR x S	2	13.802	6.901	0.3346	F<1^{ns}
		Error	25	515.598	20.624		
		Total	35	2597.959			
		CV = 21.63%					
	August-October 2015	Repetitions (blocks)	5	335.210	67.042	2.1649	0.0905^{ns}
		Watering regime	2	271.662	135.831	4.3862	0.0233*
		Species	1	145.604	145.604	4.7018	0.0399*
		WR x S	2	9.641	4.820	0.1557	F<1^{ns}
		Error	25	774.193	30.968		
		Total	35	1536.310			
		CV = 30.60%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at $P \le 0.05$ and $P \le 0.01$, respectively.

A1.6: ANOVAs for total leaf area (cm^2 $plant^{-1}$)

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	218612923.069	43722584.614	3.0902	0.0263*
		Watering regime	2	8707681.112	4353840.556	0.3077	F<1^{ns}
		Species	1	1885464.332	1885464.332	0.1333	F<1^{ns}
		WR x S	2	7199699.795	3599849.897	0.2544	F<1^{ns}
		Error	25	353718006.343	14148720.254		
		Total	35	590123774.651			
		CV = 27.23%					
	September-November 2014	Repetitions (blocks)	5	103536876.919	20707375.384	19.2881	0.0001**
		Watering regime	2	925357.518	462678.759	0.4310	F<1^{ns}
		Species	1	375916.216	375916.216	0.3502	F<1^{ns}
		WR x S	2	8173611.514	4086805.757	3.8067	0.0360*
		Error	25	26839591.924	1073583.677		
		Total	35	139851354.091			
		CV = 37.09%					
	February-April 2015	Repetitions (blocks)	5	14663485.313	2932697.063	1.7200	0.1668^{ns}
		Watering regime	2	11150126.243	5575063.122	3.2698	0.0548^{ns}
		Species	1	2849012.399	2849012.399	1.6710	0.2080^{ns}
		WR x S	2	10267288.208	5133644.104	3.0109	0.0674^{ns}
		Error	25	42625455.520	1705018.221		
		Total	35	81555367.683			
		CV = 17.70%					
Dry	April-June 2014	Repetitions (blocks)	5	44886599.351	8977319.870	12.7625	0.0001**
		Watering regime	2	15295563.906	7647781.953	10.8724	0.0004**
		Species	1	2568407.086	2568407.086	3.6513	0.0676^{ns}
		WR x S	2	4528233.440	2264116.720	3.2188	0.0570^{ns}
		Error	25	17585335.190	703413.408		
		Total	35	84864138.973			
		CV = 31.94%					
	May-July 2015	Repetitions (blocks)	5	10020574.882	2004114.976	3.4300	0.0170*
		Watering regime	2	34988623.206	17494311.603	29.9409	0.0001**
		Species	1	588641.500	588641.500	1.0074	0.3251^{ns}
		WR x S	2	111908.317	55954.159	0.0958	F<1^{ns}
		Error	25	14607345.272	584293.811		
		Total	35	60317093.176			
		CV = 35.77%					
	August-October 2015	Repetitions (blocks)	5	4248252.837	849650.567	1.8556	0.1384^{ns}
		Watering regime	2	7522188.568	3761094.284	8.2139	0.0018**
		Species	1	2812.537	2812.537	0.0061	F<1^{ns}
		WR x S	2	154656.045	77328.022	0.1689	F<1^{ns}
		Error	25	11447306.911	457892.276		
		Total	35	23375216.898			
		CV = 44.68%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A1.7: ANOVAs for aboveground biomass (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	6688.596	1337.719	2.0012	0.1133^{ns}
		Watering regime	2	600.622	300.311	0.4493	F<1^{ns}
		Species	1	467.280	467.280	0.6991	F<1^{ns}
		WR x S	2	787.670	393.835	0.5892	F<1^{ns}
		Error	25	16711.059	668.442		
		Total	35	25255.228			
		CV = 24.47%					
	September-November 2014	Repetitions (blocks)	5	11885.786	2377.157	20.3240	0.0001**
		Watering regime	2	632.416	316.208	2.7035	0.0865^{ns}
		Species	1	330.028	330.028	2.8216	0.1055^{ns}
		WR x S	2	390.202	195.101	1.6681	0.2089^{ns}
		Error	25	2924.075	116.963		
		Total	35	16162.506			
		CV = 33.01%					
	February-April 2015	Repetitions (blocks)	5	1431.376	286.275	1.7199	0.1668^{ns}
		Watering regime	2	1875.087	937.543	5.6328	0.0096**
		Species	1	69.722	69.722	0.4189	F<1^{ns}
		WR x S	2	52.687	26.343	0.1583	F<1^{ns}
		Error	25	4161.116	166.445		
		Total	35	7589.987			
		CV = 21.63%					
Dry	April-June 2014	Repetitions (blocks)	5	2454.292	490.858	10.9244	0.0001**
		Watering regime	2	847.477	423.739	9.4306	0.0009**
		Species	1	87.734	87.734	1.9526	0.1746^{ns}
		WR x S	2	429.734	214.867	4.7820	0.0174*
		Error	25	1123.308	44.932		
		Total	35	4942.546			
		CV = 25.72%					
	May-July 2015	Repetitions (blocks)	5	278.602	55.720	2.8025	0.0383*
		Watering regime	2	2459.482	1229.741	61.8508	0.0001**
		Species	1	1.323	1.323	0.0665	F<1^{ns}
		WR x S	2	24.102	12.051	0.6061	F<1^{ns}
		Error	25	497.059	19.882		
		Total	35	3260.568			
		CV = 25.13%					
	August-October 2015	Repetitions (blocks)	5	70.230	14.046	1.0584	0.4065^{ns}
		Watering regime	2	2309.895	1154.948	87.0257	0.0001**
		Species	1	5.760	5.760	0.4340	F<1^{ns}
		WR x S	2	58.522	29.261	2.2048	0.1313^{ns}
		Error	25	331.783	13.271		
		Total	35	2776.190			
		CV = 21.28%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A1.8: ANOVAs for leaf yield (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	602.758	120.552	4.0176	0.0082**
		Watering regime	2	2.484	1.242	0.0414	F<1^{ns}
		Species	1	49.000	49.000	1.6330	0.2130^{ns}
		WR x S	2	35.092	17.546	0.5847	F<1^{ns}
		Error	25	750.156	30.006		
		Total	35	1439.490			
		CV = 21.94%					
	September-November 2014	Repetitions (blocks)	5	500.145	100.029	38.1944	0.0001**
		Watering regime	2	9.129	4.564	1.7429	0.1956^{ns}
		Species	1	7.200	7.200	2.7493	0.1098^{ns}
		WR x S	2	13.296	6.648	2.5383	0.0992^{ns}
		Error	25	65.474	2.619		
		Total	35	595.243			
		CV = 27.49%					
	February-April 2015	Repetitions (blocks)	5	101.802	20.360	2.2380	0.0819^{ns}
		Watering regime	2	42.862	21.431	2.3556	0.1155^{ns}
		Species	1	1.734	1.734	0.1906	F<1^{ns}
		WR x S	2	0.327	0.164	0.0180	F<1^{ns}
		Error	25	227.442	9.098		
		Total	35	374.167			
		CV = 20.05%					
Dry	April-June 2014	Repetitions (blocks)	5	161.626	32.325	19.6679	0.0001**
		Watering regime	2	45.231	22.615	13.7600	0.0001**
		Species	1	2.064	2.064	1.2558	0.2731^{ns}
		WR x S	2	8.180	4.090	2.4886	0.1034^{ns}
		Error	25	41.089	1.644		
		Total	35	258.189			
		CV = 23.62%					
	May-July 2015	Repetitions (blocks)	5	29.096	5.819	3.0143	0.0290*
		Watering regime	2	184.880	92.440	47.8840	0.0001**
		Species	1	3.674	3.674	1.9029	0.1800^{ns}
		WR x S	2	1.816	0.908	0.4702	F<1^{ns}
		Error	25	48.263	1.931		
		Total	35	267.727			
		CV = 27.38%					
	August-October 2015	Repetitions (blocks)	5	14.171	2.834	1.5981	0.1972^{ns}
		Watering regime	2	75.061	37.530	21.1620	0.0001**
		Species	1	0.014	0.014	0.0077	F<1^{ns}
		WR x S	2	0.707	0.354	0.1994	F<1^{ns}
		Error	25	44.337	1.773		
		Total	35	134.290			
		CV = 37.54%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at $P\leq0.05$ and $P\leq0.01$, respectively.

A1.8.1: Combined analysis of variance for leaf yield

Season	Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	Transplanting date	2	6559.090	3279.545	235.8090	0.0001**
	Repetitions within TD	15	1204.706	80.314	5.7748	0.0001**
	Watering regime	2	35.697	17.849	1.2834	0.2831^{ns}
	TD x WR	4	18.777	4.694	0.3375	F<1^{ns}
	Species	1	40.333	40.333	2.9001	0.0927^{ns}
	TD x S	2	17.601	8.800	0.6328	F<1^{ns}
	WR x S	2	24.385	12.192	0.8767	F<1^{ns}
	TD x WR x S	4	24.329	6.082	0.4373	F<1^{ns}
	Error	75	1043.072	13.908		
	Total	107	8967.990			
	CV = 24.38%					
Dry	Transplanting date	2	71.911	35.955	20.3427	0.0001**
	Repetitions within TD	15	206.020	13.735	7.7707	0.0001**
	Watering regime	2	269.224	134.612	76.1600	0.0001**
	TD x WR	4	35.948	8.987	5.0846	0.0011**
	Species	1	0.044	0.044	0.0249	F<1^{ns}
	TD x S	2	5.707	2.854	1.6145	0.2058^{ns}
	WR x S	2	1.110	0.555	0.3141	F<1^{ns}
	TD x WR x S	4	9.593	2.398	1.3568	0.2570^{ns}
	Error	75	132.561	1.767		
	Total	107	732.117			
	CV = 28.39%					

df, degrees of freedom; CV, Coefficient of variation; TD, Transplanting date; WR, Watering regime; S, Species; ns, not significant; **, represent significance at $P \leq 0.01$.

A1.9: ANOVAs for stem yield (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	2111.528	422.306	3.2132	0.0224*
		Watering regime	2	188.756	94.378	0.7181	F<1^{ns}
		Species	1	18.261	18.261	0.1389	F<1^{ns}
		WR x S	2	68.913	34.457	0.2622	F<1^{ns}
		Error	25	3285.741	131.430		
		Total	35	5673.199			
		CV = 27.13%					
	September-November 2014	Repetitions (blocks)	5	997.132	199.426	20.5308	0.0001**
		Watering regime	2	20.596	10.298	1.0601	0.3615^{ns}
		Species	1	2.250	2.250	0.2316	F<1^{ns}
		WR x S	2	50.607	25.303	2.6050	0.0938^{ns}
		Error	25	242.838	9.714		
		Total	35	1313.422			
		CV = 42.82%					
	February-April 2015	Repetitions (blocks)	5	271.773	54.355	1.5065	0.2235^{ns}
		Watering regime	2	351.132	175.566	4.8660	0.0164*
		Species	1	45.788	45.788	1.2691	0.2706^{ns}
		WR x S	2	35.491	17.745	0.4918	F<1^{ns}
		Error	25	902.007	36.080		
		Total	35	1606.190			
		CV = 34.49%					
Dry	April-June 2014	Repetitions (blocks)	5	195.871	39.174	20.8664	0.0001**
		Watering regime	2	34.793	17.397	9.2664	0.0010**
		Species	1	3.306	3.306	1.7611	0.1965^{ns}
		WR x S	2	16.403	8.202	4.3686	0.0236*
		Error	25	46.935	1.877		
		Total	35	297.309			
		CV = 23.19%					
	May-July 2015	Repetitions (blocks)	5	26.907	5.381	3.0050	0.0294*
		Watering regime	2	168.665	84.332	47.0921	0.0001**
		Species	1	1.868	1.868	1.0430	0.3169^{ns}
		WR x S	2	0.261	0.130	0.0727	F<1^{ns}
		Error	25	44.770	1.791		
		Total	35	242.470			
		CV = 29.20%					
	August-October 2015	Repetitions (blocks)	5	5.131	1.026	0.9701	F<1^{ns}
		Watering regime	2	175.202	87.601	82.8083	0.0001**
		Species	1	0.380	0.380	0.3595	F<1^{ns}
		WR x S	2	0.736	0.368	0.3477	F<1^{ns}
		Error	25	26.447	1.058		
		Total	35	207.896			
		CV = 25.21%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A1.10: ANOVAs for inflorescence yield (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	397.673	79.535	0.7227	F<1^{ns}
		Watering regime	2	108.546	54.273	0.4932	F<1^{ns}
		Species	1	106.709	106.709	0.9696	F<1^{ns}
		WR x S	2	205.085	102.543	0.9318	F<1^{ns}
		Error	25	2751.227	110.049		
		Total	35	3569.240			
		CV = 27.29%					
	September-November 2014	Repetitions (blocks)	5	3046.002	609.200	13.6380	0.0001**
		Watering regime	2	314.523	157.262	3.5206	0.0450*
		Species	1	197.543	197.543	4.4224	0.0457*
		WR x S	2	92.827	46.413	1.0390	0.3686^{ns}
		Error	25	1116.730	44.669		
		Total	35	4767.626			
		CV = 34.10%					
	February-April 2015	Repetitions (blocks)	5	163.008	32.602	1.2182	0.3298^{ns}
		Watering regime	2	349.215	174.607	6.5245	0.0052**
		Species	1	8.429	8.429	0.3150	F<1^{ns}
		WR x S	2	2.308	1.154	0.0431	F<1^{ns}
		Error	25	669.048	26.762		
		Total	35	1192.008			
		CV = 19.02%					
Dry	April-June 2014	Repetitions (blocks)	5	578.618	115.724	5.9307	0.0010**
		Watering regime	2	273.616	136.808	7.0113	0.0038**
		Species	1	37.007	37.007	1.8966	0.1807^{ns}
		WR x S	2	196.202	98.101	5.0276	0.0146*
		Error	25	487.814	19.513		
		Total	35	1573.256			
		CV = 30.01%					
	May-July 2015	Repetitions (blocks)	5	63.098	12.620	2.8981	0.0338*
		Watering regime	2	535.017	267.509	61.4339	0.0001**
		Species	1	4.480	4.480	1.0289	0.3201^{ns}
		WR x S	2	16.294	8.147	1.8710	0.1749^{ns}
		Error	25	108.860	4.354		
		Total	35	727.750			
		CV = 25.75%					
	August-October 2015	Repetitions (blocks)	5	14.076	2.815	0.4485	F<1^{ns}
		Watering regime	2	699.876	349.938	55.7470	0.0001**
		Species	1	3.738	3.738	0.5954	F<1^{ns}
		WR x S	2	37.176	18.588	2.9611	0.0701^{ns}
		Error	25	156.931	6.277		
		Total	35	911.796			
		CV = 26.40%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A1.11: ANOVAs for calcium and crude protein content

Parameter	Source	df	Sum of Squares	Mean Square	F Value	Probability
Calcium	Repetitions (seasons)	3	45223805.792	15074601.931	41.8283	0.0001**
	Species	1	1986626.042	1986626.042	5.5124	0.0330*
	Watering regime	2	6047002.333	3023501.167	8.3895	0.0036**
	S x WR	2	4182308.333	2091154.167	5.8024	0.0136*
	Error	15	5405889.458	360392.631		
	Total	23	62845631.958			
	CV = 13.87%					
Crude protein	Repetitions (seasons)	3	832.407	277.469	140.5001	0.0001**
	Species	1	23.384	23.384	11.8408	0.0036**
	Watering regime	2	19.562	9.781	4.9528	0.0223*
	S x WR	2	3.224	1.612	0.8162	$F<1^{ns}$
	Error	15	29.623	1.975		
	Total	23	908.200			
	CV = 10.09%					

df, degrees of freedom; CV, Coefficient of variation; S, Species; WR, Watering regime; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A2: Results of analysis of variance (ANOVA) for Chapter 4.

A2.1: ANOVAs for panicle length (cm)

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	275.760	55.152	2.8077	0.0381*
		Watering regime	2	140.522	70.261	3.5768	0.0430*
		Species	1	100.000	100.000	5.0908	0.0330*
		WR x S	2	45.932	22.966	1.1691	0.3270^{ns}
		Error	25	491.087	19.643		
		Total	35	1053.300			
		CV = 10.69%					
	September-November 2014	Repetitions (blocks)	5	591.767	118.353	9.7983	0.0001**
		Watering regime	2	87.782	43.891	3.6337	0.0412*
		Species	1	117.361	117.361	9.7162	0.0045**
		WR x S	2	48.367	24.184	2.0021	0.1561^{ns}
		Error	25	301.973	12.079		
		Total	35	1147.250			
		CV = 10.47%					
	February-April 2015	Repetitions (blocks)	5	142.029	28.406	2.8860	0.0343*
		Watering regime	2	117.611	58.805	5.9747	0.0076**
		Species	1	45.338	45.338	4.6064	0.0417*
		WR x S	2	36.401	18.200	1.8492	0.1783^{ns}
		Error	25	246.061	9.842		
		Total	35	587.439			
		CV = 10.60%					
Dry	April-June 2014	Repetitions (blocks)	5	383.528	76.706	5.7602	0.0011**
		Watering regime	2	339.607	169.804	12.7513	0.0002**
		Species	1	93.122	93.122	6.9930	0.0139*
		WR x S	2	154.272	77.136	5.7925	0.0086**
		Error	25	332.914	13.317		
		Total	35	1303.443			
		CV = 11.90%					
	May-July 2015	Repetitions (blocks)	5	117.663	23.533	2.8201	0.0374*
		Watering regime	2	662.752	331.376	39.7119	0.0001**
		Species	1	38.647	38.647	4.6314	0.0412*
		WR x S	2	19.254	9.627	1.1537	0.3317^{ns}
		Error	25	208.612	8.344		
		Total	35	1046.927			
		CV = 13.19%					
	August-October 2015	Repetitions (blocks)	5	27.956	5.591	1.1816	0.3461^{ns}
		Watering regime	2	820.261	410.130	86.6782	0.0001**
		Species	1	22.404	22.404	4.7350	0.0392*
		WR x S	2	51.207	25.604	5.4111	0.0112*
		Error	25	118.291	4.732		
		Total	35	1040.119			
		CV = 10.56%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A2.2: ANOVAs for internode length in panicle (cm)

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	0.630	0.126	1.7436	0.1615^{ns}
		Watering regime	2	0.646	0.323	4.4711	0.0219*
		Species	1	0.355	0.355	4.9164	0.0359*
		WR x S	2	0.095	0.047	0.6573	F<1^{ns}
		Error	25	1.805	0.072		
		Total	35	3.531			
		CV = 15.30%					
	September-November 2014	Repetitions (blocks)	5	0.413	0.083	1.0438	0.4142^{ns}
		Watering regime	2	0.632	0.316	3.9878	0.0314*
		Species	1	1.868	1.868	23.5830	0.0001**
		WR x S	2	0.227	0.114	1.4345	0.2572^{ns}
		Error	25	1.980	0.079		
		Total	35	5.120			
		CV = 14.56%					
	February-April 2015	Repetitions (blocks)	5	0.028	0.006	0.2962	F<1^{ns}
		Watering regime	2	0.204	0.102	5.3812	0.0114*
		Species	1	0.147	0.147	7.7566	0.0101**
		WR x S	2	0.117	0.059	3.0938	0.0630^{ns}
		Error	25	0.474	0.019		
		Total	35	0.970			
		CV = 10.61%					
Dry	April-June 2014	Repetitions (blocks)	5	0.643	0.129	1.2049	0.3356^{ns}
		Watering regime	2	0.781	0.391	3.6634	0.0402*
		Species	1	0.715	0.715	6.7087	0.0158*
		WR x S	2	1.390	0.695	6.5163	0.0053**
		Error	25	2.666	0.107		
		Total	35	6.195			
		CV = 17.70%					
	May-July 2015	Repetitions (blocks)	5	0.701	0.140	3.8107	0.0106**
		Watering regime	2	1.524	0.762	20.6988	0.0001**
		Species	1	0.340	0.340	9.2439	0.0055**
		WR x S	2	0.017	0.009	0.2339	F<1^{ns}
		Error	25	0.920	0.037		
		Total	35	3.503			
		CV = 11.19%					
	August-October 2015	Repetitions (blocks)	5	2.868	0.574	3.1150	0.0254*
		Watering regime	2	1.529	0.764	4.1513	0.0278*
		Species	1	4.202	4.202	22.8218	0.0001**
		WR x S	2	0.527	0.263	1.4300	0.2582^{ns}
		Error	25	4.604	0.184		
		Total	35	13.730			
		CV = 21.97%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A2.3: ANOVAs for panicle straw yield (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	307.552	61.510	1.0941	0.3882^{ns}
		Watering regime	2	63.500	31.750	0.5648	F<1^{ns}
		Species	1	306.834	306.834	5.4579	0.0278*
		WR x S	2	101.576	50.788	0.9034	F<1^{ns}
		Error	25	1405.466	56.219		
		Total	35	2184.928			
		CV = 26.95%					
	September-November 2014	Repetitions (blocks)	5	1076.962	215.392	12.2440	0.0001**
		Watering regime	2	66.349	33.174	1.8858	0.1727^{ns}
		Species	1	92.801	92.801	5.2753	0.0303*
		WR x S	2	36.836	18.418	1.0470	0.3659^{ns}
		Error	25	439.791	17.592		
		Total	35	1712.739			
		CV = 38.86%					
	February-April 2015	Repetitions (blocks)	5	67.816	13.563	1.6267	0.1896^{ns}
		Watering regime	2	141.396	70.698	8.4791	0.0015**
		Species	1	17.361	17.361	2.0822	0.1614^{ns}
		WR x S	2	5.056	2.528	0.3032	F<1^{ns}
		Error	25	208.448	8.338		
		Total	35	440.076			
		CV = 20.64%					
Dry	April-June 2014	Repetitions (blocks)	5	431.101	86.220	6.9172	0.0004**
		Watering regime	2	169.984	84.992	6.8186	0.0043**
		Species	1	40.747	40.747	3.2690	0.0826^{ns}
		WR x S	2	143.574	71.787	5.7592	0.0088**
		Error	25	311.617	12.465		
		Total	35	1097.023			
		CV = 30.14%					
	May-July 2015	Repetitions (blocks)	5	36.456	7.291	3.2491	0.0214*
		Watering regime	2	310.787	155.394	69.2471	0.0001**
		Species	1	0.538	0.538	0.2396	F<1^{ns}
		WR x S	2	5.327	2.664	1.1870	0.3218^{ns}
		Error	25	56.101	2.244		
		Total	35	409.209			
		CV = 26.03%					
	August-October 2015	Repetitions (blocks)	5	8.958	1.792	0.8475	F<1^{ns}
		Watering regime	2	262.541	131.270	62.0954	0.0001**
		Species	1	0.267	0.267	0.1263	F<1^{ns}
		WR x S	2	7.154	3.577	1.6920	0.2046^{ns}
		Error	25	52.850	2.114		
		Total	35	331.770			
		CV = 25.27%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A2.4: ANOVAs for grain yield (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	65.388	13.078	1.1243	0.3732^{ns}
		Watering regime	2	19.727	9.864	0.8480	F<1^{ns}
		Species	1	51.600	51.600	4.4361	0.0454*
		WR x S	2	17.611	8.805	0.7570	F<1^{ns}
		Error	25	290.797	11.632		
		Total	35	445.123			
		CV = 32.13%					
	September-November 2014	Repetitions (blocks)	5	502.558	100.512	14.7254	0.0001**
		Watering regime	2	91.727	45.864	6.7192	0.0046**
		Species	1	19.214	19.214	2.8149	0.1059^{ns}
		WR x S	2	12.207	6.104	0.8942	F<1^{ns}
		Error	25	170.644	6.826		
		Total	35	796.350			
		CV = 29.68%					
	February-April 2015	Repetitions (blocks)	5	43.876	8.775	1.1723	0.3504^{ns}
		Watering regime	2	51.654	25.827	3.4504	0.0475*
		Species	1	49.468	49.468	6.6087	0.0165*
		WR x S	2	0.491	0.245	0.0328	F<1^{ns}
		Error	25	187.131	7.485		
		Total	35	332.619			
		CV = 20.72%					
Dry	April-June 2014	Repetitions (blocks)	5	11.962	2.392	1.9908	0.1149^{ns}
		Watering regime	2	12.487	6.244	5.1953	0.0130*
		Species	1	0.090	0.090	0.0749	F<1^{ns}
		WR x S	2	4.535	2.267	1.8868	0.1725^{ns}
		Error	25	30.044	1.202		
		Total	35	59.119			
		CV = 36.47%					
	May-July 2015	Repetitions (blocks)	5	3.957	0.791	1.6885	0.1742^{ns}
		Watering regime	2	30.252	15.126	32.2742	0.0001**
		Species	1	1.960	1.960	4.1821	0.0515^{ns}
		WR x S	2	3.395	1.697	3.6220	0.0416*
		Error	25	11.717	0.469		
		Total	35	51.280			
		CV = 29.34%					
	August-October 2015	Repetitions (blocks)	5	6.581	1.316	0.6723	F<1^{ns}
		Watering regime	2	104.921	52.460	26.7963	0.0001**
		Species	1	6.002	6.002	3.0660	0.0922^{ns}
		WR x S	2	12.022	6.011	3.0703	0.0642^{ns}
		Error	25	48.944	1.958		
		Total	35	178.470			
		CV = 37.34%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A2.4.1: Combined analysis of variance for grain yield

Season	Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	Sowing date	2	353.037	176.519	20.4109	0.0001**
	Repetitions within SD	15	612.146	40.810	4.7188	0.0001**
	Watering regime	2	144.876	72.438	8.3760	0.0005**
	SD x WR	4	18.325	4.581	0.5297	F<1 ns
	Species	1	32.111	32.111	3.7130	0.0578 ns
	SD x S	2	87.912	43.956	5.0826	0.0085**
	WR x S	2	16.696	8.348	0.9653	F<1 ns
	SD x WR x S	4	13.610	3.402	0.3934	F<1 ns
	Error	75	648.620	8.648		
	Total	107	1927.333			
	CV = 27.04%					
Dry	Sowing date	2	36.050	18.025	14.2455	0.0001**
	Repetitions within SD	15	18.628	1.242	0.9815	F<1 ns
	Watering regime	2	110.421	55.210	43.6336	0.0001**
	SD x WR	4	37.507	9.377	7.4107	0.0001**
	Species	1	0.627	0.627	0.4957	F<1 ns
	SD x S	2	7.439	3.719	2.9395	0.0590 ns
	WR x S	2	2.593	1.296	1.0246	0.3639 ns
	SD x WR x S	4	17.261	4.315	3.4103	0.0129*
	Error	75	94.899	1.265		
	Total	107	325.425			
	CV = 37.21%					

df, degrees of freedom; CV, Coefficient of variation; SD, Sowing date; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A2.5: ANOVAs for harvest index (%)

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	5	167.358	33.472	12.1526	0.0001**
		Watering regime	2	5.427	2.714	0.9852	F<1ns
		Species	1	77.734	77.734	28.2229	0.0001**
		WR x S	2	1.741	0.870	0.3160	F<1ns
		Error	25	68.857	2.754		
		Total	35	321.116			
		CV = 16.14%					
	September-November 2014	Repetitions (blocks)	5	300.582	60.116	5.4865	0.0015**
		Watering regime	2	101.401	50.700	4.6272	0.0195*
		Species	1	5.138	5.138	0.4689	F<1ns
		WR x S	2	24.801	12.400	1.1317	0.3385ns
		Error	25	273.928	10.957		
		Total	35	705.849			
		CV = 11.63%					
	February-April 2015	Repetitions (blocks)	5	99.175	19.835	2.6389	0.0477*
		Watering regime	2	19.509	9.754	1.2978	0.2909ns
		Species	1	109.900	109.900	14.6214	0.0008**
		WR x S	2	19.662	9.831	1.3080	0.2882ns
		Error	25	187.910	7.516		
		Total	35	436.156			
		CV = 12.22%					
Dry	April-June 2014	Repetitions (blocks)	5	121.395	24.279	4.7983	0.0033**
		Watering regime	2	0.457	0.229	0.0452	F<1ns
		Species	1	36.200	36.200	7.1544	0.0130*
		WR x S	2	2.221	1.110	0.2194	F<1ns
		Error	25	126.497	5.060		
		Total	35	286.770			
		CV = 18.59%					
	May-July 2015	Repetitions (blocks)	5	106.430	21.286	3.4290	0.0170*
		Watering regime	2	45.227	22.613	3.6429	0.0409*
		Species	1	66.901	66.901	10.7773	0.0030**
		WR x S	2	20.436	10.218	1.6460	0.2130ns
		Error	25	155.190	6.208		
		Total	35	394.183			
		CV = 18.17%					
	August-October 2015	Repetitions (blocks)	5	220.995	44.199	1.3079	0.2925ns
		Watering regime	2	101.288	50.644	1.4986	0.2428ns
		Species	1	160.519	160.519	4.7499	0.0389*
		WR x S	2	18.710	9.355	0.2768	F<1ns
		Error	25	844.855	33.794		
		Total	35	1346.367			
		CV = 26.12%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A2.5.1: Combined analysis of variance for harvest index

Season	Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	Sowing date	2	6171.045	3085.523	436.0587	0.0001**
	Repetitions within SD	15	567.115	37.808	5.3431	0.0001**
	Watering regime	2	25.007	12.503	1.7670	0.1779^{ns}
	SD x WR	4	101.330	25.332	3.5801	0.0100**
	Species	1	155.040	155.040	21.9109	0.0001**
	SD x S	2	37.731	18.866	2.6662	0.0761^{ns}
	WR x S	2	11.836	5.918	0.8364	F<1^{ns}
	SD x WR x S	4	34.367	8.592	1.2142	0.3119^{ns}
	Error	75	530.695	7.076		
	Total	107	7634.167			
	CV = 13.05%					
Dry	Sowing date	2	2105.709	1052.855	68.2678	0.0001**
	Repetitions within SD	15	426.138	28.409	1.8421	0.0439*
	Watering regime	2	50.088	25.044	1.6239	0.2040^{ns}
	SD x WR	4	88.598	22.150	1.4362	0.2304^{ns}
	Species	1	37.230	37.230	2.4140	0.1245^{ns}
	SD x S	2	230.144	115.072	7.4614	0.0011**
	WR x S	2	7.434	3.717	0.2410	F<1^{ns}
	SD x WR x S	4	30.653	7.663	0.4969	F<1^{ns}
	Error	75	1156.682	15.422		
	Total	107	4132.677			
	CV = 24.53%					

df, degrees of freedom; CV, Coefficient of variation; SD, Sowing date; WR, Watering regime; S, Species; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A2.6: ANOVAs for crude protein content in grain

Source	df	Sum of Squares	Mean Square	F Value	Probability
Repetitions (seasons)	3	626.755	208.918	35.7424	0.0001**
Species	1	2.761	2.761	0.4723	F<1^{ns}
Watering regime	2	7.547	3.774	0.6456	F<1^{ns}
S x WR	2	0.925	0.463	0.0792	F<1^{ns}
Error	15	87.677	5.845		
Total	23	725.666			
CV = 18.08%					

df, degrees of freedom; CV, Coefficient of variation; S, Species; WR, Watering regime; ns, not significant; **, represent significance at *P*≤0.01.

A3: Results of analysis of covariance (ANCOVA) for Chapter 5.

A3.1: ANCOVAs for leaf number

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	13526.441	6763.220	1.5794	0.2296^{ns}
		Watering regime	2	1517.056	758.528	0.1771	F<1^{ns}
		Species	1	1036.208	1036.208	0.2420	F<1^{ns}
		WR x S	2	2555.241	1277.621	0.2984	F<1^{ns}
		Harvesting intensity	1	4727.120	4727.120	1.1039	0.3053^{ns}
		WR x I	2	10101.717	5050.858	1.1795	0.3270^{ns}
		S x I	1	123.900	123.900	0.0289	F<1^{ns}
		WR x S x I	2	11941.117	5970.558	1.3943	0.2700^{ns}
		Covariate	1	51800.703	51800.703	12.6487	
		Error	21	86001.949	4095.331		
		Total	35	183331.452			
		CV = 25.01%					
	September-November 2014	Repetitions (blocks)	2	18090.172	9045.086	1.6261	0.2205^{ns}
		Watering regime	2	30793.353	15396.677	2.7680	0.0857^{ns}
		Species	1	1754.512	1754.512	0.3154	F<1^{ns}
		WR x S	2	20935.349	10467.674	1.8819	0.1771^{ns}
		Harvesting intensity	1	7813.494	7813.494	1.4047	0.2492^{ns}
		WR x I	2	1452.057	726.029	0.1305	F<1^{ns}
		S x I	1	40.433	40.433	0.0073	F<1^{ns}
		WR x S x I	2	10910.632	5455.316	0.9808	F<1^{ns}
		Covariate	1	24661.324	24661.324	4.6929	
		Error	21	110355.194	5255.009		
		Total	35	226806.520			
		CV = 20.03%					
	February-April 2015	Repetitions (blocks)	2	1073.823	536.912	0.1292	F<1
		Watering regime	2	9031.988	4515.994	1.0869	0.3555^{ns}
		Species	1	128.966	128.966	0.0310	F<1^{ns}
		WR x S	2	6325.056	3162.528	0.7612	F<1^{ns}
		Harvesting intensity	1	2073.859	2073.859	0.4991	F<1^{ns}
		WR x I	2	14730.335	7365.167	1.7727	0.1944^{ns}
		S x I	1	592.852	592.852	0.1427	F<1^{ns}
		WR x S x I	2	7587.248	3793.624	0.9131	F<1^{ns}
		Covariate	1	63616.624	63616.624	16.5323	
		Error	21	80808.254	3848.012		
		Total	35	185969.005			
		CV = 16.61%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	679.413	339.707	0.7375	F<1^{ns}
		Watering regime	2	4598.164	2299.082	4.9913	0.0168*
		Species	1	954.465	954.465	2.0721	0.1648^{ns}
		WR x S	2	2216.389	1108.194	2.4059	0.1146^{ns}
		Harvesting intensity	1	5261.276	5261.276	11.4222	0.0028**
		WR x I	2	379.239	189.619	0.4117	F<1^{ns}
		S x I	1	642.652	642.652	1.3952	0.2507^{ns}
		WR x S x I	2	2214.952	1107.476	2.4043	0.1148^{ns}
		Covariate	1	88520.426	88520.426	201.1965	
		Error	21	9239.368	439.970		
		Total	35	114706.344			
		CV = 12.89%					
	May-July 2015	Repetitions (blocks)	2	27642.606	13821.303	25.1910	0.0001**
		Watering regime	2	13667.225	6833.613	12.4551	0.0003**
		Species	1	592.324	592.324	1.0796	0.3106^{ns}
		WR x S	2	4800.090	2400.045	4.3744	0.0258*
		Harvesting intensity	1	3570.599	3570.599	6.5078	0.0186*
		WR x I	2	4304.269	2152.134	3.9225	0.0357*
		S x I	1	11.957	11.957	0.0218	F<1^{ns}
		WR x S x I	2	1487.204	743.602	1.3553	0.2795^{ns}
		Covariate	1	45495.844	45495.844	85.0762	
		Error	21	11230.080	534.766		
		Total	35	112802.198			
		CV = 11.67%					
	August-October 2015	Repetitions (blocks)	2	3689.842	1844.921	0.6114	F<1^{ns}
		Watering regime	2	24536.754	12268.377	4.0659	0.0322*
		Species	1	8127.376	8127.376	2.6935	0.1156^{ns}
		WR x S	2	864.311	432.155	0.1432	F<1^{ns}
		Harvesting intensity	1	41187.937	41187.937	13.6502	0.0013**
		WR x I	2	5994.743	2997.371	0.9934	F<1^{ns}
		S x I	1	2138.684	2138.684	0.7088	F<1^{ns}
		WR x S x I	2	6323.492	3161.746	1.0478	0.3683^{ns}
		Covariate	1	10059.041	10059.041	3.8958	
		Error	21	54222.875	2582.042		
		Total	35	157145.055			
		CV = 19.59%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A3.2: ANCOVAs for side-shoot number

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	126.366	63.183	0.8334	F<1^{ns}
		Watering regime	2	150.357	75.179	0.9916	F<1^{ns}
		Species	1	11.564	11.564	0.1525	F<1^{ns}
		WR x S	2	53.869	26.934	0.3553	F<1^{ns}
		Harvesting intensity	1	1093.441	1093.441	14.4219	0.0011**
		WR x I	2	162.392	81.196	1.0709	0.3607^{ns}
		S x I	1	7.454	7.454	0.0983	F<1^{ns}
		WR x S x I	2	167.597	83.798	1.1053	0.3496^{ns}
		Covariate	1	600.408	600.408	8.2802	
		Error	21	1522.730	72.511		
		Total	35	3896.178			
		CV = 24.42%					
	September-November 2014	Repetitions (blocks)	2	699.907	349.953	2.9146	0.0764^{ns}
		Watering regime	2	657.375	328.688	2.7375	0.0878^{ns}
		Species	1	28.638	28.638	0.2385	F<1^{ns}
		WR x S	2	305.543	152.771	1.2724	0.3009^{ns}
		Harvesting intensity	1	855.703	855.703	7.1268	0.0143*
		WR x I	2	7.180	3.590	0.0299	F<1^{ns}
		S x I	1	36.406	36.406	0.3032	F<1^{ns}
		WR x S x I	2	212.214	106.107	0.8837	F<1^{ns}
		Covariate	1	344.427	344.427	3.0363	
		Error	21	2382.125	113.435		
		Total	35	5529.518			
		CV = 22.59%					
	February-April 2015	Repetitions (blocks)	2	215.713	107.856	1.6910	0.2085^{ns}
		Watering regime	2	57.966	28.983	0.4544	F<1^{ns}
		Species	1	0.581	0.581	0.0091	F<1^{ns}
		WR x S	2	118.677	59.339	0.9303	F<1^{ns}
		Harvesting intensity	1	304.676	304.676	4.7767	0.0403*
		WR x I	2	19.559	9.780	0.1533	F<1^{ns}
		S x I	1	2.478	2.478	0.0389	F<1^{ns}
		WR x S x I	2	24.527	12.263	0.1923	F<1^{ns}
		Covariate	1	852.801	852.801	14.4361	
		Error	21	1240.560	59.074		
		Total	35	2837.538			
		CV = 18.59%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at $P≤0.05$ and $P≤0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	6.414	3.207	0.2321	F<1^{ns}
		Watering regime	2	35.045	17.522	1.2683	0.3020^{ns}
		Species	1	25.219	25.219	1.8254	0.1910^{ns}
		WR x S	2	22.182	11.091	0.8028	F<1^{ns}
		Harvesting intensity	1	325.153	325.153	23.5355	0.0001**
		WR x I	2	70.135	35.067	2.5383	0.1030^{ns}
		S x I	1	5.622	5.622	0.4069	F<1^{ns}
		WR x S x I	2	50.774	25.387	1.8376	0.1839^{ns}
		Covariate	1	1525.119	1525.119	115.5734	
		Error	21	277.118	13.196		
		Total	35	2342.781			
		CV = 16.99%					
	May-July 2015	Repetitions (blocks)	2	687.840	343.920	19.3537	0.0001**
		Watering regime	2	75.350	37.675	2.1201	0.1450^{ns}
		Species	1	2.568	2.568	0.1445	F<1^{ns}
		WR x S	2	45.197	22.598	1.2717	0.3011^{ns}
		Harvesting intensity	1	159.394	159.394	8.9697	0.0069**
		WR x I	2	21.962	10.981	0.6179	F<1^{ns}
		S x I	1	12.442	12.442	0.7002	F<1^{ns}
		WR x S x I	2	27.103	13.552	0.7626	F<1^{ns}
		Covariate	1	661.519	661.519	38.1935	
		Error	21	363.724	17.320		
		Total	35	2057.099			
		CV = 15.30%					
	August-October 2015	Repetitions (blocks)	2	69.804	34.902	0.9916	F<1^{ns}
		Watering regime	2	736.888	368.444	10.4676	0.0007**
		Species	1	77.872	77.872	2.2124	0.1518^{ns}
		WR x S	2	26.194	13.097	0.3721	F<1^{ns}
		Harvesting intensity	1	1224.080	1224.080	34.7766	0.0000**
		WR x I	2	76.714	38.357	1.0897	0.3546^{ns}
		S x I	1	0.779	0.779	0.0221	F<1^{ns}
		WR x S x I	2	57.558	28.779	0.8176	F<1^{ns}
		Covariate	1	110.742	110.742	3.6767	
		Error	21	632.522	30.120		
		Total	35	3013.153			
		CV = 16.17%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A3.3: ANCOVAs for leaf size (cm^2)

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	33.015	16.508	0.9788	$F<1^{ns}$
		Watering regime	2	29.199	14.600	0.8657	$F<1^{ns}$
		Species	1	236.378	236.378	14.0158	0.0012**
		WR x S	2	3.972	1.986	0.1178	$F<1^{ns}$
		Harvesting intensity	1	122.517	122.517	7.2645	0.0136*
		WR x I	2	10.540	5.270	0.3125	$F<1^{ns}$
		S x I	1	1.081	1.081	0.0641	$F<1^{ns}$
		WR x S x I	2	3.231	1.616	0.0958	$F<1^{ns}$
		Covariate	1	81.115	81.115	5.0290	
		Error	21	338.721	16.130		
		Total	35	859.769			
		CV = 16.19%					
	September-November 2014	Repetitions (blocks)	2	30.065	15.032	1.7775	0.1936^{ns}
		Watering regime	2	44.459	22.230	2.6286	0.0958^{ns}
		Species	1	42.315	42.315	5.0036	0.0363*
		WR x S	2	17.243	8.621	1.0194	0.0958^{ns}
		Harvesting intensity	1	37.642	37.642	4.4510	0.0471*
		WR x I	2	13.680	6.840	0.8088	$F<1^{ns}$
		S x I	1	3.732	3.732	0.4413	$F<1^{ns}$
		WR x S x I	2	3.458	1.729	0.2045	$F<1^{ns}$
		Covariate	1	166.183	166.183	20.7996	
		Error	21	167.783	7.990		
		Total	35	526.560			
		CV = 16.00%					
	February-April 2015	Repetitions (blocks)	2	33.069	16.535	2.8466	0.0806^{ns}
		Watering regime	2	44.631	22.315	3.8418	0.0379*
		Species	1	30.123	30.123	5.1860	0.0333*
		WR x S	2	32.732	16.366	2.8176	0.0824^{ns}
		Harvesting intensity	1	42.122	42.122	7.2518	0.0136*
		WR x I	2	9.942	4.971	0.8558	$F<1^{ns}$
		S x I	1	22.063	22.063	3.7984	0.0648^{ns}
		WR x S x I	2	7.430	3.715	0.6396	$F<1^{ns}$
		Covariate	1	193.068	193.068	35.8889	
		Error	21	112.972	5.380		
		Total	35	528.152			
		CV = 10.94%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at $P≤0.05$ and $P≤0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	4.074	2.037	0.3241	$F<1^{ns}$
		Watering regime	2	46.449	23.224	3.6951	0.0422*
		Species	1	63.333	63.333	10.0765	0.0046**
		WR x S	2	36.877	18.438	2.9336	0.0752^{ns}
		Harvesting intensity	1	27.525	27.525	4.3793	0.0487*
		WR x I	2	4.466	2.233	0.3553	$F<1^{ns}$
		S x I	1	0.146	0.146	0.0232	$F<1^{ns}$
		WR x S x I	2	29.436	14.718	2.3417	0.1208^{ns}
		Covariate	1	605.285	605.285	100.8226	
		Error	21	126.073	6.003		
		Total	35	943.664			
		CV = 18.27%					
	May-July 2015	Repetitions (blocks)	2	158.476	79.238	12.8957	0.0002**
		Watering regime	2	52.360	26.180	4.2607	0.0280*
		Species	1	91.148	91.148	14.8340	0.0009**
		WR x S	2	0.982	0.491	0.0799	$F<1^{ns}$
		Harvesting intensity	1	33.613	33.613	5.4704	0.0293*
		WR x I	2	32.019	16.010	2.6055	0.0975^{ns}
		S x I	1	0.050	0.050	0.0082	$F<1^{ns}$
		WR x S x I	2	0.224	0.112	0.0182	$F<1^{ns}$
		Covariate	1	92.816	92.816	15.4980	
		Error	21	125.767	5.989		
		Total	35	587.455			
		CV = 21.35%					
	August-October 2015	Repetitions (blocks)	2	0.059	0.029	0.0081	$F<1^{ns}$
		Watering regime	2	88.175	44.088	12.1112	0.0003**
		Species	1	4.593	4.593	1.2617	0.2740^{ns}
		WR x S	2	11.972	5.986	1.6444	0.2170^{ns}
		Harvesting intensity	1	0.699	0.699	0.1920	$F<1^{ns}$
		WR x I	2	3.907	1.953	0.5366	$F<1^{ns}$
		S x I	1	0.459	0.459	0.1260	$F<1^{ns}$
		WR x S x I	2	5.843	2.921	0.8025	$F<1^{ns}$
		Covariate	1	4.529	4.529	1.4540	
		Error	21	65.416	3.115		
		Total	35	185.652			
		CV = 16.76%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at $P≤0.05$ and $P≤0.01$, respectively.

A3.4: ANCOVAs for total leaf area (cm^2 plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	17162595.8	8581297.9	1.1513	0.3354^{ns}
		Watering regime	2	2255234.9	1127617.5	0.1513	F<1^{ns}
		Species	1	18541939.1	18541939.1	2.4877	0.1297^{ns}
		WR x S	2	2051539.0	1025769.5	0.1376	F<1^{ns}
		Harvesting intensity	1	28688305.6	28688305.6	3.8490	0.0632^{ns}
		WR x I	2	7334929.8	3667464.9	0.4920	F<1^{ns}
		S x I	1	60449.5	60449.5	0.0081	F<1^{ns}
		WR x S x I	2	7130701.0	3565350.5	0.4783	F<1^{ns}
		Covariate	1	70123401.4	70123401.4	9.8371	
		Error	21	149697000.8	7128428.6		
		Total	35	303046096.9			
		CV = 40.38%					
	September-November 2014	Repetitions (blocks)	2	7114232.5	3557116.2	0.9037	F<1^{ns}
		Watering regime	2	24860452.0	12430226.0	3.1579	0.0632^{ns}
		Species	1	2807357.3	2807357.3	0.7132	F<1^{ns}
		WR x S	2	17159001.4	8579500.7	2.1796	0.1380^{ns}
		Harvesting intensity	1	14981446.3	14981446.3	3.8061	0.0645^{ns}
		WR x I	2	784979.6	392489.8	0.0997	F<1^{ns}
		S x I	1	274795.4	274795.4	0.0698	F<1^{ns}
		WR x S x I	2	546244.2	273122.1	0.0694	F<1^{ns}
		Covariate	1	48078260.0	48078260.0	12.9287	
		Error	21	78093403.7	3718733.5		
		Total	35	194700172.4			
		CV = 29.06%					
	February-April 2015	Repetitions (blocks)	2	10367321.5	5183660.8	1.2014	0.3206^{ns}
		Watering regime	2	19796562.2	9898281.1	2.2941	0.1256^{ns}
		Species	1	2092486.2	2092486.2	0.4850	F<1^{ns}
		WR x S	2	1891723.3	945861.6	0.2192	F<1^{ns}
		Harvesting intensity	1	1075.3	1075.3	0.0002	F<1^{ns}
		WR x I	2	12481727.5	6240863.8	1.4464	0.2579^{ns}
		S x I	1	1499178.5	1499178.5	0.3475	F<1^{ns}
		WR x S x I	2	12093729.4	6046864.7	1.4015	0.2683^{ns}
		Covariate	1	97187732.4	97187732.4	24.3211	
		Error	21	83916694.7	3996033.1		
		Total	35	241328231.1			
		CV = 24.32%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	61030.5	30515.2	0.0728	F<1^{ns}
		Watering regime	2	9762193.8	4881096.9	11.6508	0.0004**
		Species	1	3677622.0	3677622.0	8.7782	0.0074**
		WR x S	2	2448746.7	1224373.4	2.9225	0.0759^{ns}
		Harvesting intensity	1	1947494.6	1947494.6	4.6485	0.0428*
		WR x I	2	203147.1	101573.6	0.2424	F<1^{ns}
		S x I	1	13428.0	13428.0	0.0321	F<1^{ns}
		WR x S x I	2	2639356.2	1319678.1	3.1500	0.0636^{ns}
		Covariate	1	71670402.2	71670402.2	179.1011	
		Error	21	8403513.9	400167.3		
		Total	35	100826935.0			
		CV = 25.17%					
	May-July 2015	Repetitions (blocks)	2	14041011.1	7020505.5	15.9946	0.0001**
		Watering regime	2	8547717.6	4273858.8	9.7370	0.0010**
		Species	1	2525572.0	2525572.0	5.7539	0.0258*
		WR x S	2	657173.3	328586.6	0.7486	F<1^{ns}
		Harvesting intensity	1	5188715.8	5188715.8	11.8213	0.0025**
		WR x I	2	3502645.5	1751322.7	3.9900	0.0340*
		S x I	1	9291.4	9291.4	0.0212	F<1^{ns}
		WR x S x I	2	42059.4	21029.7	0.0479	F<1^{ns}
		Covariate	1	17722631.7	17722631.7	41.4262	
		Error	21	8984064.3	427812.6		
		Total	35	61220882.0			
		CV = 26.34%					
	August-October 2015	Repetitions (blocks)	2	372133.6	186066.8	0.2322	F<1^{ns}
		Watering regime	2	17826689.5	8913344.7	11.1233	0.0005**
		Species	1	54333.5	54333.5	0.0678	F<1^{ns}
		WR x S	2	750388.4	375194.2	0.4682	F<1^{ns}
		Harvesting intensity	1	6931315.7	6931315.7	8.6499	0.0078**
		WR x I	2	3366754.0	1683377.0	2.1008	0.1473^{ns}
		S x I	1	9001.8	9001.8	0.0112	F<1^{ns}
		WR x S x I	2	1960968.1	980484.1	1.2236	0.3143^{ns}
		Covariate	1	2494534.8	2494534.8	3.6379	
		Error	21	14399893.8	685709.2		
		Total	35	48166013.1			
		CV = 29.01%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at P≤0.05 and P≤0.01, respectively.

A3.5: ANCOVAs for aboveground biomass (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	622.635	311.317	2.8374	0.0811^{ns}
		Watering regime	2	211.419	105.710	0.9635	F<1^{ns}
		Species	1	258.169	258.169	2.3530	0.1400^{ns}
		WR x S	2	12.180	6.090	0.0555	F<1^{ns}
		Harvesting intensity	1	1609.035	1609.035	14.6651	0.0010**
		WR x I	2	130.306	65.153	0.5938	F<1^{ns}
		S x I	1	6.546	6.546	0.0597	F<1^{ns}
		WR x S x I	2	246.557	123.278	1.1236	0.3439^{ns}
		Covariate	1	614.215	614.215	5.8534	
		Error	21	2203.587	104.933		
		Total	35	5914.649			
		CV = 28.95%					
	September-November 2014	Repetitions (blocks)	2	48.304	24.152	0.3313	F<1^{ns}
		Watering regime	2	304.743	152.371	2.0903	0.1486^{ns}
		Species	1	105.651	105.651	1.4494	0.2420^{ns}
		WR x S	2	294.322	147.161	2.0188	0.1578^{ns}
		Harvesting intensity	1	342.160	342.160	4.6939	0.0419*
		WR x I	2	73.534	36.767	0.5044	F<1^{ns}
		S x I	1	47.718	47.718	0.6546	F<1^{ns}
		WR x S x I	2	11.705	5.853	0.0803	F<1^{ns}
		Covariate	1	1109.720	1109.720	16.1140	
		Error	21	1446.206	68.867		
		Total	35	3784.063			
		CV = 29.17%					
	February-April 2015	Repetitions (blocks)	2	252.573	126.287	4.8786	0.0182*
		Watering regime	2	208.740	104.370	4.0320	0.0330*
		Species	1	32.068	32.068	1.2388	0.2783^{ns}
		WR x S	2	18.015	9.007	0.3480	F<1^{ns}
		Harvesting intensity	1	9.936	9.936	0.3839	F<1^{ns}
		WR x I	2	41.832	20.916	0.8080	F<1^{ns}
		S x I	1	6.530	6.530	0.2522	F<1^{ns}
		WR x S x I	2	52.925	26.463	1.0223	0.3770^{ns}
		Covariate	1	920.955	920.955	38.4144	
		Error	21	503.458	23.974		
		Total	35	2047.032			
		CV = 20.22%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	1.730	0.865	0.2552	F<1^{ns}
		Watering regime	2	34.573	17.287	5.1004	0.0157*
		Species	1	24.892	24.892	7.3442	0.0131*
		WR x S	2	18.133	9.067	2.6751	0.0923^{ns}
		Harvesting intensity	1	41.926	41.926	12.3700	0.0020**
		WR x I	2	0.648	0.324	0.0956	F<1^{ns}
		S x I	1	1.351	1.351	0.3986	F<1^{ns}
		WR x S x I	2	17.102	8.551	2.5230	0.1042^{ns}
		Covariate	1	584.277	584.277	180.4800	
		Error	21	67.984	3.237		
		Total	35	792.616			
		CV = 22.30%					
	May-July 2015	Repetitions (blocks)	2	108.268	54.134	11.1911	0.0005**
		Watering regime	2	78.562	39.281	8.1206	0.0024**
		Species	1	12.982	12.982	2.6839	0.1163^{ns}
		WR x S	2	27.141	13.571	2.8054	0.0832^{ns}
		Harvesting intensity	1	52.753	52.753	10.9056	0.0034**
		WR x I	2	30.266	15.133	3.1284	0.0647^{ns}
		S x I	1	2.752	2.752	0.5689	F<1^{ns}
		WR x S x I	2	12.711	6.356	1.3139	0.2900^{ns}
		Covariate	1	183.642	183.642	38.9507	
		Error	21	99.009	4.715		
		Total	35	608.086			
		CV = 24.71%					
	August-October 2015	Repetitions (blocks)	2	5.786	2.893	0.2830	F<1^{ns}
		Watering regime	2	193.550	96.775	9.4676	0.0012**
		Species	1	0.641	0.641	0.0627	F<1^{ns}
		WR x S	2	6.529	3.264	0.3194	F<1^{ns}
		Harvesting intensity	1	89.703	89.703	8.7758	0.0074**
		WR x I	2	15.617	7.808	0.7639	F<1^{ns}
		S x I	1	7.103	7.103	0.6949	F<1^{ns}
		WR x S x I	2	5.046	2.523	0.2468	F<1^{ns}
		Covariate	1	18.360	18.360	2.0991	
		Error	21	183.685	8.747		
		Total	35	526.020			
		CV = 27.86%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A3.6: ANCOVAs for leaf yield (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	116.941	58.470	2.3141	0.1235^{ns}
		Watering regime	2	54.741	27.370	1.0832	0.3567^{ns}
		Species	1	98.806	98.806	3.9105	0.0613^{ns}
		WR x S	2	8.237	4.118	0.1630	F<1^{ns}
		Harvesting intensity	1	134.734	134.734	5.3325	0.0312*
		WR x I	2	34.560	17.280	0.6839	F<1^{ns}
		S x I	1	0.738	0.738	0.0292	F<1^{ns}
		WR x S x I	2	73.430	36.715	1.4531	0.2564^{ns}
		Covariate	1	140.285	140.285	5.8054	
		Error	21	507.460	24.165		
		Total	35	1169.932			
		CV = 26.34%					
	September-November 2014	Repetitions (blocks)	2	12.094	6.047	0.4106	F<1^{ns}
		Watering regime	2	88.352	44.176	2.9999	0.0715^{ns}
		Species	1	29.053	29.053	1.9729	0.1748^{ns}
		WR x S	2	56.668	28.334	1.9241	0.1709^{ns}
		Harvesting intensity	1	10.237	10.237	0.6951	F<1^{ns}
		WR x I	2	4.653	2.327	0.1580	F<1^{ns}
		S x I	1	4.163	4.163	0.2827	F<1^{ns}
		WR x S x I	2	8.517	4.258	0.2892	F<1^{ns}
		Covariate	1	210.178	210.178	15.1072	
		Error	21	292.160	13.912		
		Total	35	716.075			
		CV = 25.66%					
	February-April 2015	Repetitions (blocks)	2	79.312	39.656	4.6971	0.0206*
		Watering regime	2	80.425	40.213	4.7630	0.0197*
		Species	1	16.632	16.632	1.9700	0.1751^{ns}
		WR x S	2	11.809	5.904	0.6994	F<1^{ns}
		Harvesting intensity	1	0.110	0.110	0.0131	F<1^{ns}
		WR x I	2	26.550	13.275	1.5724	0.2310^{ns}
		S x I	1	3.386	3.386	0.4010	F<1^{ns}
		WR x S x I	2	9.452	4.726	0.5598	F<1^{ns}
		Covariate	1	246.524	246.524	31.5278	
		Error	21	164.204	7.819		
		Total	35	638.404			
		CV = 18.18%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	1.644	0.822	0.8207	F<1 ns
		Watering regime	2	9.856	4.928	4.9216	0.0177*
		Species	1	12.459	12.459	12.4425	0.0020**
		WR x S	2	6.289	3.145	3.1404	0.0641 ns
		Harvesting intensity	1	5.356	5.356	5.3491	0.0310*
		WR x I	2	0.724	0.362	0.3617	F<1 ns
		S x I	1	0.395	0.395	0.3948	F<1 ns
		WR x S x I	2	6.242	3.121	3.1169	0.0653 ns
		Covariate	1	214.557	214.557	224.3309	
		Error	21	20.085	0.956		
		Total	35	277.607			
		CV = 18.91%					
	May-July 2015	Repetitions (blocks)	2	40.685	20.343	15.3258	0.0001**
		Watering regime	2	28.025	14.013	10.5568	0.0007**
		Species	1	6.393	6.393	4.8163	0.0396*
		WR x S	2	9.637	4.818	3.6302	0.0443*
		Harvesting intensity	1	9.093	9.093	6.8506	0.0161*
		WR x I	2	8.232	4.116	3.1010	0.0661 ns
		S x I	1	1.105	1.105	0.8327	F<1 ns
		WR x S x I	2	2.706	1.353	1.0195	0.3780 ns
		Covariate	1	72.333	72.333	55.9109	
		Error	21	27.168	1.294		
		Total	35	205.377			
		CV = 20.50%					
	August-October 2015	Repetitions (blocks)	2	5.343	2.671	0.7951	F<1 ns
		Watering regime	2	80.308	40.154	11.9520	0.0003**
		Species	1	0.208	0.208	0.0619	F<1 ns
		WR x S	2	1.336	0.668	0.1988	F<1 ns
		Harvesting intensity	1	17.429	17.429	5.1878	0.0333*
		WR x I	2	5.123	2.562	0.7624	F<1 ns
		S x I	1	1.590	1.590	0.4732	F<1 ns
		WR x S x I	2	1.416	0.708	0.2107	F<1 ns
		Covariate	1	2.619	2.619	0.9111	
		Error	21	60.373	2.875		
		Total	35	175.745			
		CV = 27.14%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A3.7: ANCOVAs for stem yield (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	199.958	99.979	3.2567	0.0586^{ns}
		Watering regime	2	51.076	25.538	0.8319	F<1^{ns}
		Species	1	37.547	37.547	1.2230	0.2813^{ns}
		WR x S	2	0.864	0.432	0.0141	F<1^{ns}
		Harvesting intensity	1	812.358	812.358	26.4616	0.0001**
		WR x I	2	30.925	15.462	0.5037	F<1^{ns}
		S x I	1	2.911	2.911	0.0948	F<1^{ns}
		WR x S x I	2	52.118	26.059	0.8488	F<1^{ns}
		Covariate	1	167.390	167.390	5.7012	
		Error	21	616.570	29.360		
		Total	35	1971.717			
		CV = 32.39%					
	September-November 2014	Repetitions (blocks)	2	30.802	15.401	0.6355	F<1^{ns}
		Watering regime	2	65.732	32.866	1.3563	0.2793^{ns}
		Species	1	23.898	23.898	0.9862	F<1^{ns}
		WR x S	2	92.918	46.459	1.9172	0.1719^{ns}
		Harvesting intensity	1	234.031	234.031	9.6578	0.0053**
		WR x I	2	41.400	20.700	0.8542	F<1^{ns}
		S x I	1	23.691	23.691	0.9777	F<1^{ns}
		WR x S x I	2	0.653	0.326	0.0135	F<1^{ns}
		Covariate	1	354.002	354.002	15.4629	
		Error	21	480.767	22.894		
		Total	35	1347.894			
		CV = 34.40%					
	February-April 2015	Repetitions (blocks)	2	51.792	25.896	3.9100	0.0360*
		Watering regime	2	30.458	15.229	2.2994	0.1250^{ns}
		Species	1	2.511	2.511	0.3792	F<1^{ns}
		WR x S	2	0.911	0.456	0.0688	F<1^{ns}
		Harvesting intensity	1	12.139	12.139	1.8329	0.1902^{ns}
		WR x I	2	2.631	1.315	0.1986	F<1^{ns}
		S x I	1	0.512	0.512	0.0773	F<1^{ns}
		WR x S x I	2	18.373	9.186	1.3871	0.2718^{ns}
		Covariate	1	214.510	214.510	34.9711	
		Error	21	128.812	6.134		
		Total	35	462.649			
		CV = 28.01%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	0.362	0.181	0.1902	F<1^{ns}
		Watering regime	2	7.524	3.762	3.9502	0.0350*
		Species	1	2.130	2.130	2.2366	0.1497^{ns}
		WR x S	2	3.104	1.552	1.6299	0.2198^{ns}
		Harvesting intensity	1	17.311	17.311	18.1773	0.0003**
		WR x I	2	0.003	0.001	0.0015	F<1^{ns}
		S x I	1	0.285	0.285	0.2990	F<1^{ns}
		WR x S x I	2	2.736	1.368	1.4363	0.2602^{ns}
		Covariate	1	90.708	90.708	99.7167	
		Error	21	19.103	0.910		
		Total	35	143.266			
		CV = 32.96%					
	May-July 2015	Repetitions (blocks)	2	17.417	8.709	6.2579	0.0074**
		Watering regime	2	12.947	6.473	4.6518	0.0213*
		Species	1	1.155	1.155	0.8300	F<1^{ns}
		WR x S	2	5.268	2.634	1.8927	0.1755^{ns}
		Harvesting intensity	1	18.043	18.043	12.9652	0.0017**
		WR x I	2	8.075	4.038	2.9014	0.0772^{ns}
		S x I	1	0.369	0.369	0.2652	F<1^{ns}
		WR x S x I	2	4.289	2.144	1.5409	0.2374^{ns}
		Covariate	1	25.468	25.468	18.7763	
		Error	21	28.484	1.356		
		Total	35	121.515			
		CV = 35.95%					
	August-October 2015	Repetitions (blocks)	2	0.127	0.063	0.0298	F<1^{ns}
		Watering regime	2	24.716	12.358	5.8044	0.0098**
		Species	1	0.119	0.119	0.0558	F<1^{ns}
		WR x S	2	2.035	1.017	0.4778	F<1^{ns}
		Harvesting intensity	1	28.051	28.051	13.1755	0.0016**
		WR x I	2	2.883	1.442	0.6771	F<1^{ns}
		S x I	1	1.972	1.972	0.9262	F<1^{ns}
		WR x S x I	2	1.446	0.723	0.3396	F<1^{ns}
		Covariate	1	7.110	7.110	3.9025	
		Error	21	38.260	1.822		
		Total	35	106.719			
		CV = 30.90%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A3.8: ANCOVAs for leaf/stem ratio

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	0.109	0.055	4.9116	0.0178*
		Watering regime	2	0.026	0.013	1.1727	0.3290^{ns}
		Species	1	0.021	0.021	1.8401	0.1893^{ns}
		WR x S	2	0.039	0.020	1.7591	0.1966^{ns}
		Harvesting intensity	1	1.874	1.874	168.1074	0.0001**
		WR x I	2	0.030	0.015	1.3448	0.2821^{ns}
		S x I	1	0.004	0.004	0.3591	F<1^{ns}
		WR x S x I	2	0.029	0.014	1.2999	0.2936^{ns}
		Covariate	1	0.057	0.057	5.3120	
		Error	21	0.224	0.011		
		Total	35	2.413			
		CV = 8.66%					
	September-November 2014	Repetitions (blocks)	2	0.210	0.105	6.4384	0.0066**
		Watering regime	2	0.023	0.011	0.6952	F<1^{ns}
		Species	1	0.006	0.006	0.3654	F<1^{ns}
		WR x S	2	0.055	0.027	1.6798	0.2105^{ns}
		Harvesting intensity	1	1.264	1.264	77.6069	0.0001**
		WR x I	2	0.157	0.079	4.8197	0.0189*
		S x I	1	0.002	0.002	0.1352	F<1^{ns}
		WR x S x I	2	0.054	0.027	1.6725	0.2118^{ns}
		Covariate	1	0.325	0.325	21.1030	
		Error	21	0.323	0.015		
		Total	35	2.419			
		CV = 10.84%					
	February-April 2015	Repetitions (blocks)	2	0.004	0.002	0.0190	F<1^{ns}
		Watering regime	2	0.022	0.011	0.1034	F<1^{ns}
		Species	1	0.104	0.104	0.9904	F<1^{ns}
		WR x S	2	0.099	0.050	0.4716	F<1^{ns}
		Harvesting intensity	1	0.773	0.773	7.3327	0.0132*
		WR x I	2	0.014	0.007	0.0686	F<1^{ns}
		S x I	1	0.004	0.004	0.0388	F<1^{ns}
		WR x S x I	2	0.072	0.036	0.3423	F<1^{ns}
		Covariate	1	2.278	2.278	23.3254	
		Error	21	2.051	0.098		
		Total	35	5.421			
		CV = 16.06%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at P≤0.05 and P≤0.01, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	0.173	0.086	0.6406	F<1^ns
		Watering regime	2	0.107	0.053	0.3964	F<1^ns
		Species	1	0.000	0.000	0.0010	F<1^ns
		WR x S	2	0.054	0.027	0.2012	F<1^ns
		Harvesting intensity	1	5.109	5.109	37.9373	0.0001**
		WR x I	2	0.152	0.076	0.5660	F<1^ns
		S x I	1	0.183	0.183	1.3563	0.2572^ns
		WR x S x I	2	0.003	0.001	0.0094	F<1^ns
		Covariate	1	2.395	2.395	18.6161	
		Error	21	2.701	0.129		
		Total	35	10.877			
		CV = 17.71%					
	May-July 2015	Repetitions (blocks)	2	0.251	0.126	1.1356	0.3402^ns
		Watering regime	2	0.015	0.008	0.0693	F<1^ns
		Species	1	0.352	0.352	3.1835	0.0888^ns
		WR x S	2	0.267	0.133	1.2072	0.3190^ns
		Harvesting intensity	1	0.646	0.646	5.8408	0.0248*
		WR x I	2	0.875	0.437	3.9543	0.0349*
		S x I	1	0.029	0.029	0.2621	F<1^ns
		WR x S x I	2	0.415	0.208	1.8784	0.1776^ns
		Covariate	1	0.002	0.002	0.0208	
		Error	21	2.263	0.108		
		Total	35	5.115			
		CV = 17.49%					
	August-October 2015	Repetitions (blocks)	2	0.275	0.138	2.4002	0.1151^ns
		Watering regime	2	0.208	0.104	1.8189	0.1868^ns
		Species	1	0.011	0.011	0.1899	F<1^ns
		WR x S	2	0.082	0.041	0.7177	F<1^ns
		Harvesting intensity	1	0.823	0.823	14.3615	0.0011**
		WR x I	2	0.008	0.004	0.0692	F<1^ns
		S x I	1	0.020	0.020	0.3525	F<1^ns
		WR x S x I	2	0.064	0.032	0.5583	F<1^ns
		Covariate	1	0.257	0.257	5.2359	
		Error	21	1.030	0.049		
		Total	35	2.778			
		CV = 14.76%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; I, Harvesting intensity; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A3.9: Combined analysis of variance for calcium and crude protein content

Parameter	Source	df	Sum of Squares	Mean Square	F Value	Probability
Calcium	Harvesting time	1	23735731.510	23735731.510	39.4773	0.0001**
	Repetitions in HT	6	249469811.146	41578301.858	69.1530	0.0001**
	Species	1	121055.010	121055.010	0.2013	F<1^{ns}
	HT x S	1	1598826.260	1598826.260	2.6592	0.1077^{ns}
	Watering regime	2	2906121.333	1453060.667	2.4167	0.0971^{ns}
	HT x WR	2	2910082.583	1455041.292	2.4200	0.0968^{ns}
	S x WR	2	449961.083	224980.542	0.3742	F<1^{ns}
	HT x S x WR	2	707252.583	353626.292	0.5882	F<1^{ns}
	Harvesting intensity	1	11245.010	11245.010	0.0187	F<1^{ns}
	HT x I	1	442409.260	442409.260	0.7358	F<1^{ns}
	S x I	1	939510.510	939510.510	1.5626	0.2157^{ns}
	HT x S x I	1	184713.760	184713.760	0.3072	F<1^{ns}
	WR x I	2	1072041.083	536020.542	0.8915	F<1^{ns}
	HT x WR x I	2	1373470.583	686735.292	1.1422	0.3253^{ns}
	S x WR x I	2	48836.083	24418.042	0.0406	F<1^{ns}
	HT x S x WR x I	2	747504.333	373752.167	0.6216	F<1^{ns}
	Error	66	39682555.104	601250.835		
	Total	95	326401127.240			
	CV = 34.82%					
Crude protein	Harvesting time	1	743.818	743.818	60.0949	0.0001**
	Repetitions in HT	6	2257.893	376.315	30.4035	0.0001**
	Species	1	36.039	36.039	2.9117	0.0926^{ns}
	HT x S	1	0.778	0.778	0.0628	F<1^{ns}
	Watering regime	2	24.808	12.404	1.0021	0.3726^{ns}
	HT x WR	2	21.853	10.927	0.8828	F<1^{ns}
	S x WR	2	53.414	26.707	2.1577	0.1237^{ns}
	HT x S x WR	2	34.350	17.175	1.3876	0.2569^{ns}
	Harvesting intensity	1	0.217	0.217	0.0175	F<1^{ns}
	HT x I	1	27.714	27.714	2.2390	0.1393^{ns}
	S x I	1	5.236	5.236	0.4230	F<1^{ns}
	HT x S x I	1	4.438	4.438	0.3585	F<1^{ns}
	WR x I	2	2.875	1.438	0.1161	F<1^{ns}
	HT x WR x I	2	17.116	8.558	0.6914	F<1^{ns}
	S x WR x I	2	6.179	3.090	0.2496	F<1^{ns}
	HT x S x WR x I	2	1.389	0.694	0.0561	F<1^{ns}
	Error	66	816.907	12.377		
	Total	95	4055.022			
	CV = 22.69%					

df, degrees of freedom; CV, Coefficient of variation; HT, Harvesting time; S, Species; WR, Watering regime; I, Harvesting intensity; ns, not significant; **, represent significance at $P \leq 0.01$.

A4: Results of analysis of covariance (ANCOVA) for Chapter 6.

A4.1: ANCOVAs for leaf number

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	41448.442	20724.221	3.3835	0.0532^{ns}
		Watering regime	2	29477.561	14738.780	2.4063	0.1146^{ns}
		Species	1	19313.656	19313.656	3.1532	0.0903^{ns}
		WR x S	2	27486.151	13743.076	2.2437	0.1309^{ns}
		Harvesting frequency	1	26647.936	26647.936	4.3506	0.0494*
		WR x F	2	13146.218	6573.109	1.0731	0.3600^{ns}
		S x F	1	4318.953	4318.953	0.7051	F<1^{ns}
		WR x S x F	2	2429.900	1214.950	0.1984	F<1^{ns}
		Covariate	1	75608.250	75608.250	13.5211	
		Error	21	117429.418	5591.877		
		Total	35	357306.485			
		CV = 30.62%					
	September-November 2014	Repetitions (blocks)	2	15359.593	7679.796	2.9026	0.0771^{ns}
		Watering regime	2	56229.478	28114.739	10.6261	0.0006**
		Species	1	889.307	889.307	0.3361	F<1^{ns}
		WR x S	2	1371.008	685.504	0.2591	F<1^{ns}
		Harvesting frequency	1	37150.306	37150.306	14.0411	0.0012**
		WR x F	2	2046.468	1023.234	0.3867	F<1^{ns}
		S x F	1	8372.916	8372.916	3.1646	0.0897^{ns}
		WR x S x F	2	2842.867	1421.433	0.5372	F<1^{ns}
		Covariate	1	8500.540	8500.540	3.3419	
		Error	21	53415.366	2543.589		
		Total	35	186177.849			
		CV = 14.13%					
	February-April 2015	Repetitions (blocks)	2	2501.720	1250.860	0.7008	F<1^{ns}
		Watering regime	2	33320.304	16660.152	9.3344	0.0013**
		Species	1	4496.497	4496.497	2.5193	0.1274^{ns}
		WR x S	2	5276.525	2638.263	1.4782	0.2508^{ns}
		Harvesting frequency	1	27925.283	27925.283	15.6460	0.0007**
		WR x F	2	1958.839	979.420	0.5488	F<1^{ns}
		S x F	1	0.648	0.648	0.0004	F<1^{ns}
		WR x S x F	2	6299.931	3149.966	1.7649	0.1957^{ns}
		Covariate	1	1.113	1.113	0.0006	
		Error	21	36246.412	1726.020		
		Total	35	118027.272			
		CV = 13.17%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at P≤0.05 and P≤0.01, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	13536.145	6768.072	5.6230	0.0111*
		Watering regime	2	8686.388	4343.194	3.6084	0.0450*
		Species	1	418.722	418.722	0.3479	F<1^{ns}
		WR x S	2	1716.275	858.137	0.7130	F<1^{ns}
		Harvesting frequency	1	1155.873	1155.873	0.9603	F<1^{ns}
		WR x F	2	2002.868	1001.434	0.8320	F<1^{ns}
		S x F	1	1862.549	1862.549	1.5474	0.2272^{ns}
		WR x S x F	2	290.707	145.354	0.1208	F<1^{ns}
		Covariate	1	73123.140	73123.140	62.2054	
		Error	21	24685.752	1175.512		
		Total	35	127478.419			
		CV = 20.98%					
	May-July 2015	Repetitions (blocks)	2	18316.733	9158.367	14.2991	0.0001**
		Watering regime	2	8338.152	4169.076	6.5093	0.0063**
		Species	1	966.182	966.182	1.5085	0.2330^{ns}
		WR x S	2	5062.953	2531.476	3.9524	0.0349*
		Harvesting frequency	1	264.315	264.315	0.4127	F<1^{ns}
		WR x F	2	546.450	273.225	0.4266	F<1^{ns}
		S x F	1	167.359	167.359	0.2613	F<1^{ns}
		WR x S x F	2	5383.829	2691.914	4.2029	0.0292*
		Covariate	1	28852.788	28852.788	47.7009	
		Error	21	12702.233	604.868		
		Total	35	80600.994			
		CV = 12.26%					
	August-October 2015	Repetitions (blocks)	2	6209.498	3104.749	1.0530	0.3666^{ns}
		Watering regime	2	41713.706	20856.853	7.0738	0.0045**
		Species	1	16085.600	16085.600	5.4556	0.0295*
		WR x S	2	2315.507	1157.753	0.3927	F<1^{ns}
		Harvesting frequency	1	21460.727	21460.727	7.2787	0.0135*
		WR x F	2	8309.552	4154.776	1.4091	0.2665^{ns}
		S x F	1	6705.272	6705.272	2.2742	0.1464^{ns}
		WR x S x F	2	10768.419	5384.210	1.8261	0.1857^{ns}
		Covariate	1	7885.511	7885.511	3.2415	
		Error	21	51086.482	2432.690		
		Total	35	172540.274			
		CV = 17.76%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A4.2: ANCOVAs for side-shoot number

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	460.158	230.079	2.3938	0.1157^{ns}
		Watering regime	2	377.411	188.706	1.9634	0.1653^{ns}
		Species	1	127.362	127.362	1.3251	0.2626^{ns}
		WR x S	2	135.999	68.000	0.7075	F<1^{ns}
		Harvesting frequency	1	1922.831	1922.831	20.0058	0.0002**
		WR x F	2	1.264	0.632	0.0066	F<1^{ns}
		S x F	1	187.248	187.248	1.9482	0.1774^{ns}
		WR x S x F	2	0.766	0.383	0.0040	F<1^{ns}
		Covariate	1	1051.303	1051.303	11.9812	
		Error	21	1842.675	87.746		
		Total	35	6107.017			
		CV = 26.61%					
	September-November 2014	Repetitions (blocks)	2	229.571	114.785	2.0445	0.1544^{ns}
		Watering regime	2	584.681	292.340	5.2069	0.0146*
		Species	1	2.396	2.396	0.0427	F<1^{ns}
		WR x S	2	225.995	112.998	2.0126	0.1586^{ns}
		Harvesting frequency	1	1980.505	1980.505	35.2751	0.0000**
		WR x F	2	130.381	65.191	1.1611	0.3324^{ns}
		S x F	1	44.397	44.397	0.7908	F<1^{ns}
		WR x S x F	2	20.405	10.202	0.1817	F<1^{ns}
		Covariate	1	167.746	167.746	3.1078	
		Error	21	1133.477	53.975		
		Total	35	4519.554			
		CV = 15.54%					
	February-April 2015	Repetitions (blocks)	2	135.271	67.635	1.6513	0.2157^{ns}
		Watering regime	2	411.317	205.658	5.0212	0.0165*
		Species	1	11.129	11.129	0.2717	F<1^{ns}
		WR x S	2	92.178	46.089	1.1253	0.3434^{ns}
		Harvesting frequency	1	1222.367	1222.367	29.8445	0.0001**
		WR x F	2	397.981	198.990	4.8584	0.0184*
		S x F	1	7.968	7.968	0.1945	F<1^{ns}
		WR x S x F	2	42.350	21.175	0.5170	F<1^{ns}
		Covariate	1	83.737	83.737	2.1141	
		Error	21	831.783	39.609		
		Total	35	3236.081			
		CV = 17.75%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	404.143	202.071	8.9607	0.0015**
		Watering regime	2	59.091	29.545	1.3102	0.2909^{ns}
		Species	1	0.022	0.022	0.0010	F<1^{ns}
		WR x S	2	6.771	3.385	0.1501	F<1^{ns}
		Harvesting frequency	1	145.145	145.145	6.4363	0.0192*
		WR x F	2	73.828	36.914	1.6369	0.2184^{ns}
		S x F	1	0.113	0.113	0.0050	F<1^{ns}
		WR x S x F	2	17.255	8.628	0.3826	F<1^{ns}
		Covariate	1	878.721	878.721	39.8984	
		Error	21	462.504	22.024		
		Total	35	2047.593			
		CV = 21.88%					
	May-July 2015	Repetitions (blocks)	2	390.617	195.308	15.6515	0.0001**
		Watering regime	2	208.185	104.092	8.3417	0.0022**
		Species	1	97.052	97.052	7.7775	0.0110*
		WR x S	2	38.206	19.103	1.5309	0.2395^{ns}
		Harvesting frequency	1	91.407	91.407	7.3251	0.0132*
		WR x F	2	32.881	16.440	1.3175	0.2890^{ns}
		S x F	1	2.247	2.247	0.1801	F<1^{ns}
		WR x S x F	2	104.919	52.460	4.2040	0.0291*
		Covariate	1	451.597	451.597	38.3207	
		Error	21	247.478	11.785		
		Total	35	1664.589			
		CV = 12.39%					
	August-October 2015	Repetitions (blocks)	2	114.274	57.137	1.3740	0.2749^{ns}
		Watering regime	2	342.204	171.102	4.1147	0.0311*
		Species	1	121.443	121.443	2.9205	0.1022^{ns}
		WR x S	2	129.490	64.745	1.5570	0.2341^{ns}
		Harvesting frequency	1	784.894	784.894	18.8753	0.0003**
		WR x F	2	271.034	135.517	3.2589	0.0585^{ns}
		S x F	1	122.571	122.571	2.9476	0.1007^{ns}
		WR x S x F	2	217.899	108.950	2.6200	0.0964^{ns}
		Covariate	1	65.208	65.208	1.9006	
		Error	21	720.492	34.309		
		Total	35	2889.509			
		CV = 16.92%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A4.3: ANCOVAs for leaf size (cm^2)

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	55.824	27.912	1.1246	0.3436^{ns}
		Watering regime	2	236.267	118.134	4.7595	0.0197*
		Species	1	363.987	363.987	14.6649	0.0010**
		WR x S	2	152.070	76.035	3.0634	0.0680^{ns}
		Harvesting frequency	1	265.760	265.760	10.7073	0.0036**
		WR x F	2	14.480	7.240	0.2917	F<1^{ns}
		S x F	1	0.571	0.571	0.0230	F<1^{ns}
		WR x S x F	2	5.841	2.920	0.1177	F<1^{ns}
		Covariate	1	195.200	195.200	8.6144	
		Error	21	475.852	22.660		
		Total	35	1765.852			
		CV = 16.67%					
	September-November 2014	Repetitions (blocks)	2	172.261	86.131	8.5989	0.0019**
		Watering regime	2	236.931	118.466	11.8271	0.0004**
		Species	1	56.079	56.079	5.5987	0.0277*
		WR x S	2	27.938	13.969	1.3946	0.2700^{ns}
		Harvesting frequency	1	74.893	74.893	7.4770	0.0124*
		WR x F	2	17.063	8.531	0.8517	F<1^{ns}
		S x F	1	0.552	0.552	0.0551	F<1^{ns}
		WR x S x F	2	3.751	1.875	0.1872	F<1^{ns}
		Covariate	1	181.722	181.722	18.8716	
		Error	21	202.218	9.629		
		Total	35	973.408			
		CV = 14.98%					
	February-April 2015	Repetitions (blocks)	2	59.371	29.685	2.5593	0.1012^{ns}
		Watering regime	2	80.052	40.026	3.4507	0.0506*
		Species	1	50.732	50.732	4.3737	0.0488*
		WR x S	2	43.104	21.552	1.8581	0.1807^{ns}
		Harvesting frequency	1	111.620	111.620	9.6230	0.0054**
		WR x F	2	34.655	17.328	1.4939	0.2474^{ns}
		S x F	1	1.205	1.205	0.1039	F<1^{ns}
		WR x S x F	2	11.850	5.925	0.5108	F<1^{ns}
		Covariate	1	20.629	20.629	1.8390	
		Error	21	235.561	11.217		
		Total	35	648.779			
		CV = 13.89%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	150.978	75.489	9.4095	0.0012**
		Watering regime	2	58.318	29.159	3.6346	0.0441*
		Species	1	57.122	57.122	7.1201	0.0144*
		WR x S	2	12.744	6.372	0.7943	F<1^{ns}
		Harvesting frequency	1	91.832	91.832	11.4466	0.0028**
		WR x F	2	7.471	3.735	0.4656	F<1^{ns}
		S x F	1	0.061	0.061	0.0076	F<1^{ns}
		WR x S x F	2	1.326	0.663	0.0826	F<1^{ns}
		Covariate	1	730.296	730.296	93.2074	
		Error	21	164.539	7.835		
		Total	35	1274.687			
		CV = 18.24%					
	May-July 2015	Repetitions (blocks)	2	118.688	59.344	10.0802	0.0009**
		Watering regime	2	111.266	55.633	9.4497	0.0012**
		Species	1	29.519	29.519	5.0140	0.0361*
		WR x S	2	19.792	9.896	1.6809	0.2103^{ns}
		Harvesting frequency	1	85.807	85.807	14.5751	0.0010**
		WR x F	2	2.226	1.113	0.1890	F<1^{ns}
		S x F	1	2.870	2.870	0.4874	F<1^{ns}
		WR x S x F	2	10.579	5.290	0.8985	F<1^{ns}
		Covariate	1	126.101	126.101	22.6805	
		Error	21	116.757	5.560		
		Total	35	623.605			
		CV = 17.20%					
	August-October 2015	Repetitions (blocks)	2	15.885	7.943	3.7087	0.0417*
		Watering regime	2	40.303	20.152	9.4097	0.0012**
		Species	1	1.374	1.374	0.6414	F<1^{ns}
		WR x S	2	11.362	5.681	2.6528	0.0939^{ns}
		Harvesting frequency	1	25.081	25.081	11.7114	0.0026**
		WR x F	2	1.379	0.689	0.3219	F<1^{ns}
		S x F	1	2.366	2.366	1.1048	0.3052^{ns}
		WR x S x F	2	10.692	5.346	2.4963	0.1065^{ns}
		Covariate	1	50.469	50.469	28.5626	
		Error	21	37.106	1.767		
		Total	35	196.017			
		CV = 11.12%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A4.4: ANCOVAs for total leaf area (cm^2 $plant^{-1}$)

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	63851708.1	31925854.0	3.3345	0.0553^{ns}
		Watering regime	2	71902082.4	35951041.2	3.7549	0.0404*
		Species	1	84408237.2	84408237.2	8.8160	0.0073**
		WR x S	2	63907224.0	31953612.0	3.3374	0.0551^{ns}
		Harvesting frequency	1	1719354.2	1719354.2	0.1796	$F<1^{ns}$
		WR x F	2	16985294.0	8492647.0	0.8870	$F<1^{ns}$
		S x F	1	4131206.2	4131206.2	0.4315	$F<1^{ns}$
		WR x S x F	2	190655.9	95327.9	0.0100	$F<1^{ns}$
		Covariate	1	127357664.6	127357664.6	14.5703	
		Error	21	183558732.3	8740892.0		
		Total	35	618012158.9			
		CV = 40.78%					
	September-November 2014	Repetitions (blocks)	2	25056587.8	12528293.9	3.0646	0.0680^{ns}
		Watering regime	2	89670631.9	44835315.9	10.9672	0.0005**
		Species	1	5924456.3	5924456.3	1.4492	0.2421^{ns}
		WR x S	2	5630263.8	2815131.9	0.6886	$F<1^{ns}$
		Harvesting frequency	1	716276.1	716276.1	0.1752	$F<1^{ns}$
		WR x F	2	2605833.5	1302916.8	0.3187	$F<1^{ns}$
		S x F	1	2513832.1	2513832.1	0.6149	$F<1^{ns}$
		WR x S x F	2	2698332.5	1349166.2	0.3300	$F<1^{ns}$
		Covariate	1	40580896.8	40580896.8	10.3255	
		Error	21	82533552.7	3930169.2		
		Total	35	257930663.5			
		CV = 26.06%					
	February-April 2015	Repetitions (blocks)	2	4331747.5	2165873.8	0.8819	$F<1^{ns}$
		Watering regime	2	46046342.4	23023171.2	9.3741	0.0012**
		Species	1	809829.1	809829.1	0.3297	$F<1^{ns}$
		WR x S	2	13013564.5	6506782.3	2.6493	0.0942^{ns}
		Harvesting frequency	1	490051.8	490051.8	0.1995	$F<1^{ns}$
		WR x F	2	1440028.2	720014.1	0.2932	$F<1^{ns}$
		S x F	1	408879.3	408879.3	0.1665	$F<1^{ns}$
		WR x S x F	2	8082873.1	4041436.6	1.6455	0.2168^{ns}
		Covariate	1	1447382.5	1447382.5	0.6094	
		Error	21	49877793.3	2375133.0		
		Total	35	125948491.7			
		CV = 20.21%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P\leq0.05$ and $P\leq0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	13502844.1	6751422.0	6.6185	0.0059**
		Watering regime	2	12089953.5	6044976.7	5.9260	0.0091**
		Species	1	4281575.0	4281575.0	4.1973	0.0532*
		WR x S	2	588102.6	294051.3	0.2883	F<1 ns
		Harvesting frequency	1	2798407.3	2798407.3	2.7433	0.1125 ns
		WR x F	2	1992707.1	996353.5	0.9767	F<1 ns
		S x F	1	846876.6	846876.6	0.8302	F<1 ns
		WR x S x F	2	226444.7	113222.3	0.1110	F<1 ns
		Covariate	1	78582275.0	78582275.0	78.8782	
		Error	21	20921206.6	996247.9		
		Total	35	135830392.5			
		CV = 35.11%					
	May-July 2015	Repetitions (blocks)	2	12634394.7	6317197.4	11.0120	0.0005**
		Watering regime	2	14373164.9	7186582.5	12.5275	0.0003**
		Species	1	2959441.2	2959441.2	5.1588	0.0338*
		WR x S	2	2187367.6	1093683.8	1.9065	0.1735 ns
		Harvesting frequency	1	3257551.0	3257551.0	5.6785	0.0267*
		WR x F	2	37374.8	18687.4	0.0326	F<1 ns
		S x F	1	138205.9	138205.9	0.2409	F<1 ns
		WR x S x F	2	2006809.4	1003404.7	1.7491	0.1983 ns
		Covariate	1	18143017.5	18143017.5	33.4887	
		Error	21	11377077.9	541765.6		
		Total	35	67114404.9			
		CV = 24.74%					
	August-October 2015	Repetitions (blocks)	2	3161680.6	1580840.3	1.7369	0.2004 ns
		Watering regime	2	20781304.1	10390652.1	11.4164	0.0004**
		Species	1	841830.8	841830.8	0.9249	F<1 ns
		WR x S	2	499435.8	249717.9	0.2744	F<1 ns
		Harvesting frequency	1	441728.1	441728.1	0.4853	F<1 ns
		WR x F	2	584410.3	292205.1	0.3211	F<1 ns
		S x F	1	1163004.6	1163004.6	1.2778	0.2711 ns
		WR x S x F	2	2679201.7	1339600.9	1.4718	0.2522 ns
		Covariate	1	9086424.7	9086424.7	12.1000	
		Error	21	15769847.3	750945.1		
		Total	35	55008868.0			
		CV = 25.21%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P \le 0.05$ and $P \le 0.01$, respectively.

A4.5: ANCOVAs for aboveground biomass (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	2378.227	1189.113	6.0497	0.0084**
		Watering regime	2	2175.670	1087.835	5.5344	0.0117*
		Species	1	1836.252	1836.252	9.3420	0.0060**
		WR x S	2	2069.922	1034.961	5.2654	0.0140*
		Harvesting frequency	1	328.580	328.580	1.6717	0.2101^{ns}
		WR x F	2	501.853	250.926	1.2766	0.2998^{ns}
		S x F	1	20.370	20.370	0.1036	F<1^{ns}
		WR x S x F	2	151.266	75.633	0.3848	F<1^{ns}
		Covariate	1	3134.821	3134.821	17.4693	
		Error	21	3768.389	179.447		
		Total	35	16365.35			
		CV = 27.77%					
	September-November 2014	Repetitions (blocks)	2	22.523	11.261	0.0612	F<1^{ns}
		Watering regime	2	2923.682	1461.841	7.9474	0.0027**
		Species	1	394.426	394.426	2.1443	0.1579^{ns}
		WR x S	2	74.386	37.193	0.2022	F<1^{ns}
		Harvesting frequency	1	337.562	337.562	1.8352	0.1899^{ns}
		WR x F	2	420.411	210.205	1.1428	0.3380^{ns}
		S x F	1	159.151	159.151	0.8652	F<1^{ns}
		WR x S x F	2	106.352	53.176	0.2891	F<1^{ns}
		Covariate	1	2232.932	2232.932	12.6274	
		Error	21	3713.472	176.832		
		Total	35	10384.897			
		CV = 34.54%					
	February-April 2015	Repetitions (blocks)	2	220.865	110.432	4.8469	0.0186*
		Watering regime	2	922.845	461.423	20.2518	0.0001**
		Species	1	81.715	81.715	3.5865	0.0721^{ns}
		WR x S	2	98.804	49.402	2.1682	0.1393^{ns}
		Harvesting frequency	1	41.993	41.993	1.8431	0.1890^{ns}
		WR x F	2	19.116	9.558	0.4195	F<1^{ns}
		S x F	1	0.110	0.110	0.0048	F<1^{ns}
		WR x S x F	2	93.571	46.785	2.0534	0.1533^{ns}
		Covariate	1	74.292	74.292	3.3717	
		Error	21	462.709	22.034		
		Total	35	2016.020			
		CV = 18.55%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	121.061	60.530	7.8745	0.0028**
		Watering regime	2	58.015	29.007	3.7737	0.0398*
		Species	1	29.851	29.851	3.8834	0.0621^{ns}
		WR x S	2	15.490	7.745	1.0076	0.3821^{ns}
		Harvesting frequency	1	0.541	0.541	0.0704	F<1^{ns}
		WR x F	2	21.336	10.668	1.3878	0.2716^{ns}
		S x F	1	2.049	2.049	0.2666	F<1^{ns}
		WR x S x F	2	1.245	0.622	0.0810	F<1^{ns}
		Covariate	1	624.175	624.175	83.1433	
		Error	21	157.652	7.507		
		Total	35	1031.415			
		CV = 30.26%					
	May-July 2015	Repetitions (blocks)	2	127.678	63.839	7.8203	0.0029**
		Watering regime	2	139.993	69.997	8.5746	0.0019**
		Species	1	52.884	52.884	6.4783	0.0188*
		WR x S	2	20.583	10.291	1.2607	0.3040^{ns}
		Harvesting frequency	1	0.216	0.216	0.0264	F<1^{ns}
		WR x F	2	2.088	1.044	0.1279	F<1^{ns}
		S x F	1	3.571	3.571	0.4374	F<1^{ns}
		WR x S x F	2	29.984	14.992	1.8365	0.1841^{ns}
		Covariate	1	226.454	226.454	29.3741	
		Error	21	161.896	7.709		
		Total	35	765.347			
		CV = 25.99%					
	August-October 2015	Repetitions (blocks)	2	9.547	4.774	0.3875	F<1^{ns}
		Watering regime	2	355.132	177.566	14.4154	0.0001**
		Species	1	12.614	12.614	1.0240	0.3231^{ns}
		WR x S	2	9.307	4.653	0.3778	F<1^{ns}
		Harvesting frequency	1	8.377	8.377	0.6801	F<1^{ns}
		WR x F	2	7.202	3.601	0.2923	F<1^{ns}
		S x F	1	5.191	5.191	0.4214	F<1^{ns}
		WR x S x F	2	32.772	16.386	1.3303	0.2858^{ns}
		Covariate	1	41.111	41.111	4.0451	
		Error	21	213.426	10.163		
		Total	35	694.679			
		CV = 24.14%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A4.6: ANCOVAs for leaf yield (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	346.437	173.218	4.4812	0.0239*
		Watering regime	2	353.045	176.523	4.5667	0.0226*
		Species	1	459.774	459.774	11.8945	0.0024**
		WR x S	2	294.131	147.066	3.8046	0.0389*
		Harvesting frequency	1	1.068	1.068	0.0276	F<1^{ns}
		WR x F	2	88.921	44.460	1.1502	0.3357^{ns}
		S x F	1	17.356	17.356	0.4490	F<1^{ns}
		WR x S x F	2	3.927	1.964	0.0508	F<1^{ns}
		Covariate	1	595.694	595.694	16.8804	
		Error	21	741.072	35.289		
		Total	35	2901.425			
		CV = 27.79%					
	September-November 2014	Repetitions (blocks)	2	11.215	5.608	0.2154	F<1^{ns}
		Watering regime	2	589.195	294.598	11.3154	0.0005**
		Species	1	115.893	115.893	4.4514	0.0470*
		WR x S	2	21.532	10.766	0.4135	F<1^{ns}
		Harvesting frequency	1	0.095	0.095	0.0037	F<1^{ns}
		WR x F	2	27.672	13.836	0.5314	F<1^{ns}
		S x F	1	20.190	20.190	0.7755	F<1^{ns}
		WR x S x F	2	20.031	10.015	0.3847	F<1^{ns}
		Covariate	1	194.074	194.074	7.7539	
		Error	21	525.611	25.029		
		Total	35	1525.508			
		CV = 30.50%					
	February-April 2015	Repetitions (blocks)	2	37.423	18.711	3.2923	0.0571^{ns}
		Watering regime	2	224.477	112.239	19.7484	0.0001**
		Species	1	28.014	28.014	4.9291	0.0375*
		WR x S	2	35.877	17.938	3.1563	0.0633^{ns}
		Harvesting frequency	1	2.732	2.732	0.4806	F<1^{ns}
		WR x F	2	0.803	0.401	0.0706	F<1^{ns}
		S x F	1	2.687	2.687	0.4727	F<1^{ns}
		WR x S x F	2	27.741	13.871	2.4405	0.1114^{ns}
		Covariate	1	1.276	1.276	0.2321	
		Error	21	115.420	5.496		
		Total	35	476.450			
		CV = 15.91%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	36.792	18.396	6.8755	0.0050**
		Watering regime	2	19.179	9.589	3.5840	0.0458*
		Species	1	12.738	12.738	4.7609	0.0406*
		WR x S	2	3.980	1.990	0.7438	F<1^{ns}
		Harvesting frequency	1	0.721	0.721	0.2694	F<1^{ns}
		WR x F	2	7.245	3.623	1.3539	0.2799^{ns}
		S x F	1	0.897	0.897	0.3351	F<1^{ns}
		WR x S x F	2	0.311	0.155	0.0581	F<1^{ns}
		Covariate	1	166.606	166.606	63.7586	
		Error	21	54.875	2.613		
		Total	35	303.344			
		CV = 30.34%					
	May-July 2015	Repetitions (blocks)	2	38.123	19.062	11.0890	0.0005**
		Watering regime	2	30.966	15.483	9.0071	0.0015**
		Species	1	18.539	18.539	10.7846	0.0035**
		WR x S	2	4.960	2.480	1.4427	0.2588^{ns}
		Harvesting frequency	1	0.629	0.629	0.3659	F<1^{ns}
		WR x F	2	0.997	0.499	0.2900	F<1^{ns}
		S x F	1	1.256	1.256	0.7305	F<1^{ns}
		WR x S x F	2	8.395	4.198	2.4420	0.1113^{ns}
		Covariate	1	60.363	60.363	37.1830	
		Error	21	34.091	1.623		
		Total	35	198.319			
		CV = 20.68%					
	August-October 2015	Repetitions (blocks)	2	6.726	3.363	1.3284	0.2863^{ns}
		Watering regime	2	64.555	32.277	12.7487	0.0002**
		Species	1	4.115	4.115	1.6254	0.2163^{ns}
		WR x S	2	2.629	1.314	0.5191	F<1^{ns}
		Harvesting frequency	1	2.530	2.530	0.9992	F<1^{ns}
		WR x F	2	2.633	1.316	0.5199	F<1^{ns}
		S x F	1	1.669	1.669	0.6592	F<1^{ns}
		WR x S x F	2	9.875	4.937	1.9501	0.1672^{ns}
		Covariate	1	8.378	8.378	4.0106	
		Error	21	43.868	2.089		
		Total	35	146.978			
		CV = 21.14%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A4.7: ANCOVAs for stem yield (g plant^{-1})

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	930.058	465.029	7.0924	0.0044**
		Watering regime	2	784.258	392.129	5.9806	0.0088**
		Species	1	458.353	458.353	6.9906	0.0152*
		WR x S	2	812.664	406.332	6.1972	0.0077**
		Harvesting frequency	1	367.114	367.114	5.5991	0.0277*
		WR x F	2	171.828	85.914	1.3103	0.2909^{ns}
		S x F	1	0.121	0.121	0.0018	F<1^{ns}
		WR x S x F	2	107.072	53.536	0.8165	F<1^{ns}
		Covariate	1	997.463	997.463	16.6636	
		Error	21	1257.038	59.859		
		Total	35	5885.969			
		CV = 28.80%					
	September-November 2014	Repetitions (blocks)	2	5.209	2.605	0.0342	F<1^{ns}
		Watering regime	2	900.891	450.445	5.9226	0.0091**
		Species	1	82.715	82.715	1.0876	0.3089^{ns}
		WR x S	2	25.110	12.555	0.1651	F<1^{ns}
		Harvesting frequency	1	326.303	326.303	4.2903	0.0508*
		WR x F	2	249.095	124.547	1.6376	0.2183^{ns}
		S x F	1	65.969	65.969	0.8674	F<1^{ns}
		WR x S x F	2	38.669	19.334	0.2542	F<1^{ns}
		Covariate	1	1110.414	1110.414	15.1868	
		Error	21	1535.455	73.117		
		Total	35	4339.830			
		CV = 38.71%					
	February-April 2015	Repetitions (blocks)	2	76.460	38.230	5.0680	0.0160*
		Watering regime	2	238.187	119.093	15.7879	0.0001**
		Species	1	14.038	14.038	1.8610	0.1870^{ns}
		WR x S	2	15.851	7.926	1.0507	0.3674^{ns}
		Harvesting frequency	1	66.146	66.146	8.7687	0.0075**
		WR x F	2	12.389	6.195	0.8212	F<1^{ns}
		S x F	1	3.881	3.881	0.5145	F<1^{ns}
		WR x S x F	2	24.487	12.244	1.6231	0.2211^{ns}
		Covariate	1	56.098	56.098	7.6900	
		Error	21	153.192	7.295		
		Total	35	660.729			
		CV = 25.54%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P≤0.05$ and $P≤0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	24.597	12.299	7.9300	0.0027**
		Watering regime	2	10.788	5.394	3.4779	0.0496*
		Species	1	3.589	3.589	2.3144	0.1431^{ns}
		WR x S	2	4.031	2.016	1.2996	0.2937^{ns}
		Harvesting frequency	1	0.013	0.013	0.0083	F<1^{ns}
		WR x F	2	3.720	1.860	1.1994	0.3212^{ns}
		S x F	1	0.235	0.235	0.1515	F<1^{ns}
		WR x S x F	2	0.419	0.209	0.1350	F<1^{ns}
		Covariate	1	145.827	145.827	96.2767	
		Error	21	31.808	1.515		
		Total	35	225.027			
		CV = 33.03%					
	May-July 2015	Repetitions (blocks)	2	26.815	13.408	5.2064	0.0146*
		Watering regime	2	40.091	20.045	7.7839	0.0030**
		Species	1	8.800	8.800	3.4172	0.0787^{ns}
		WR x S	2	5.479	2.739	1.0638	0.3630^{ns}
		Harvesting frequency	1	0.108	0.108	0.0419	F<1^{ns}
		WR x F	2	0.374	0.187	0.0726	F<1^{ns}
		S x F	1	0.591	0.591	0.2297	F<1^{ns}
		WR x S x F	2	6.650	3.325	1.2912	0.2959^{ns}
		Covariate	1	52.985	52.985	21.7863	
		Error	21	51.073	2.432		
		Total	35	192.966			
		CV = 34.50%					
	August-October 2015	Repetitions (blocks)	2	3.847	1.923	0.4564	F<1^{ns}
		Watering regime	2	117.112	58.556	13.8944	0.0001**
		Species	1	2.319	2.319	0.5504	F<1^{ns}
		WR x S	2	2.155	1.078	0.2557	F<1^{ns}
		Harvesting frequency	1	20.114	20.114	4.7727	0.0404**
		WR x F	2	11.025	5.513	1.3080	0.2915^{ns}
		S x F	1	0.973	0.973	0.2309	F<1^{ns}
		WR x S x F	2	6.962	3.481	0.8260	F<1^{ns}
		Covariate	1	12.371	12.371	3.5579	
		Error	21	73.021	3.477		
		Total	35	249.899			
		CV = 29.28%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

A4.8: ANCOVAs for leaf/stem ratio

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Rainy	January-March 2014	Repetitions (blocks)	2	0.090	0.045	6.4794	0.0064**
		Watering regime	2	0.022	0.011	1.6155	0.2225^{ns}
		Species	1	0.049	0.049	7.0179	0.0150*
		WR x S	2	0.032	0.016	2.2879	0.1262^{ns}
		Harvesting frequency	1	0.315	0.315	45.4085	0.0001**
		WR x F	2	0.001	0.000	0.0489	F<1^{ns}
		S x F	1	0.002	0.002	0.2525	F<1^{ns}
		WR x S x F	2	0.038	0.019	2.7207	0.0890^{ns}
		Covariate	1	0.006	0.006	0.9131	
		Error	21	0.133	0.006		
		Total	35	0.688			
		CV = 9.61%					
	September-November 2014	Repetitions (blocks)	2	0.050	0.025	1.6020	0.2252^{ns}
		Watering regime	2	0.041	0.021	1.3394	0.2835^{ns}
		Species	1	0.080	0.080	5.1865	0.0333*
		WR x S	2	0.040	0.020	1.2850	0.2975^{ns}
		Harvesting frequency	1	0.432	0.432	27.8883	0.0001**
		WR x F	2	0.033	0.016	1.0651	0.3626^{ns}
		S x F	1	0.006	0.006	0.3586	F<1^{ns}
		WR x S x F	2	0.020	0.010	0.6429	F<1^{ns}
		Covariate	1	0.356	0.356	23.9206	
		Error	21	0.313	0.015		
		Total	35	1.371			
		CV = 14.96%					
	February-April 2015	Repetitions (blocks)	2	0.166	0.083	2.2386	0.1314^{ns}
		Watering regime	2	0.620	0.310	8.3529	0.0021**
		Species	1	0.080	0.080	2.1631	0.1562^{ns}
		WR x S	2	0.025	0.013	0.3389	F<1^{ns}
		Harvesting frequency	1	1.631	1.631	43.9626	0.0001**
		WR x F	2	0.007	0.004	0.0990	F<1^{ns}
		S x F	1	0.157	0.157	4.2247	0.0525^{ns}
		WR x S x F	2	0.282	0.141	3.8004	0.0390*
		Covariate	1	0.651	0.651	18.1323	
		Error	21	0.754	0.036		
		Total	35	4.373			
		CV = 12.36%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Seasons		Source	df	Sum of Squares	Mean Square	F Value	Probability
Dry	April-June 2014	Repetitions (blocks)	2	0.396	0.198	4.7578	0.0198*
		Watering regime	2	0.049	0.024	0.5871	F<1^{ns}
		Species	1	0.029	0.029	0.6964	F<1^{ns}
		WR x S	2	0.346	0.173	4.1563	0.0301*
		Harvesting frequency	1	0.014	0.014	0.3253	F<1^{ns}
		WR x F	2	0.009	0.005	0.1132	F<1^{ns}
		S x F	1	0.004	0.004	0.0883	F<1^{ns}
		WR x S x F	2	0.024	0.012	0.2898	F<1^{ns}
		Covariate	1	2.483	2.483	61.0641	
		Error	21	0.854	0.041		
		Total	35	4.208			
		CV = 12.25%					
	May-July 2015	Repetitions (blocks)	2	0.848	0.424	4.9170	0.0177*
		Watering regime	2	0.123	0.061	0.7102	F<1^{ns}
		Species	1	0.002	0.002	0.0240	F<1^{ns}
		WR x S	2	0.193	0.096	1.1159	0.3463^{ns}
		Harvesting frequency	1	0.170	0.170	1.9719	0.1749^{ns}
		WR x F	2	0.001	0.001	0.0069	F<1^{ns}
		S x F	1	0.163	0.163	1.8865	0.1841^{ns}
		WR x S x F	2	0.135	0.067	0.7816	F<1^{ns}
		Covariate	1	1.132	1.132	13.8980	
		Error	21	1.711	0.081		
		Total	35	4.478			
		CV = 18.07%					
	August-October 2015	Repetitions (blocks)	2	0.334	0.167	8.7322	0.0017**
		Watering regime	2	0.202	0.101	5.2845	0.0138*
		Species	1	0.001	0.001	0.0657	F<1^{ns}
		WR x S	2	0.054	0.027	1.4129	0.2656^{ns}
		Harvesting frequency	1	0.774	0.774	40.5087	0.0001**
		WR x F	2	0.068	0.034	1.7863	0.1921^{ns}
		S x F	1	0.016	0.016	0.8206	F<1^{ns}
		WR x S x F	2	0.014	0.007	0.3731	F<1^{ns}
		Covariate	1	0.009	0.009	0.6010	
		Error	21	0.331	0.016		
		Total	35	1.803			
		CV = 10.97%					

df, degrees of freedom; CV, Coefficient of variation; WR, Watering regime; S, Species; F, Harvesting frequency; ns, not significant; * and **, represent significance at *P*≤0.05 and *P*≤0.01, respectively.

A4.9: Combined analysis of variance for calcium and crude protein content

Parameter	Source	df	Sum of Squares	Mean Square	F Value	Probability
Calcium	Harvesting time	1	4429004.2	4429004.2	4.5208	0.0372*
	Repetitions in HT	6	313047814.0	52174635.7	53.2554	0.0001**
	Species	1	176645.0	176645.0	0.1803	F<1^{ns}
	HT x S	1	424270.0	424270.0	0.4331	F<1^{ns}
	Watering regime	2	174666.9	87333.4	0.0891	F<1^{ns}
	HT x WR	2	856379.4	428189.7	0.4371	F<1^{ns}
	S x WR	2	2803034.6	1401517.3	1.4305	0.2465^{ns}
	HT x S x WR	2	2173816.4	1086908.2	1.1094	0.3358^{ns}
	Harvesting frequency	1	67098.4	67098.4	0.0685	F<1^{ns}
	HT x F	1	1187705.0	1187705.0	1.2123	0.2749^{ns}
	S x F	1	293930.7	293930.7	0.3000	F<1^{ns}
	HT x S x F	1	1318828.2	1318828.2	1.3461	0.2501^{ns}
	WR x F	2	687437.3	343718.7	0.3508	F<1^{ns}
	HT x WR x F	2	1543076.6	771538.3	0.7875	F<1^{ns}
	S x WR x F	2	1858480.6	929240.3	0.9485	F<1^{ns}
	HT x S x WR x F	2	1955915.9	977957.9	0.9982	F<1^{ns}
	Error	66	64660546.0	979705.2		
	Total	95	397658649.3			
	CV = 42.78%					
Crude protein	Harvesting time	1	147.758	147.758	10.6172	0.0018**
	Repetitions in HT	6	1662.046	277.008	19.9045	0.0001**
	Species	1	60.325	60.325	4.3347	0.0412*
	HT x S	1	7.426	7.426	0.5336	F<1^{ns}
	Watering regime	2	43.876	21.938	1.5764	0.2144^{ns}
	HT x WR	2	64.172	32.086	2.3056	0.1077^{ns}
	S x WR	2	58.616	29.308	2.1059	0.1298^{ns}
	HT x S x WR	2	19.582	9.791	0.7036	F<1^{ns}
	Harvesting frequency	1	1.063	1.063	0.0764	F<1^{ns}
	HT x F	1	51.188	51.188	3.6781	0.0595^{ns}
	S x F	1	36.878	36.878	2.6499	0.1083^{ns}
	HT x S x F	1	0.863	0.863	0.0620	F<1^{ns}
	WR x F	2	61.053	30.527	2.1935	0.1196^{ns}
	HT x WR x F	2	0.253	0.127	0.0091	
	S x WR x F	2	6.906	3.453	0.2481	
	HT x S x WR x F	2	4.836	2.418	0.1737	
	Error	66	918.511	13.917		
	Total	95	3145.352			
	CV = 23.73%					

df, degrees of freedom; CV, Coefficient of variation; HT, Harvesting time; S, Species; WR, Watering regime; F, Harvesting frequency; ns, not significant; **, represent significance at $P \leq 0.01$.